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INFECTION EARLY IN LIFE AND RISK OF NON-AFFECTIVE PSYCHOSIS

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INFECTION EARLY IN LIFE AND RISK OF NON-AFFECTIVE PSYCHOSIS

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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ABSTRACT

In light of the increasing, but insufficient, evidence that various infections and immunological processes during brain development are associated with psychotic disorder, the aim of this thesis was to investigate infection during foetal life and childhood on the risk of developing non-affective psychosis later in life. We conducted two population-based birth cohort studies using linkages to several nationwide registers as well as two studies on a subset of cases and controls in which biological samples were examined.

In Study I, the association between specific chronic maternal infections during pregnancy and non-affective psychoses in the offspring was assessed. For that purpose we analysed levels of antibodies in neonatal dried blood samples (NDBS) directed at four neurotropic agents; *Toxoplasma gondii* (*T. gondii*), cytomegalovirus (CMV) as well as herpes simplex virus type 1 and 2. Maternal *T. gondii* and CMV infections were found to be associated with schizophrenia.

In Study II, chronic maternal infection during pregnancy, neonatal immune markers, and subsequent non-affective psychosis were investigated. NDBS were analysed to determine levels of antibodies directed at *T. gondii* and three herpes viruses as well as levels of acute phase proteins (APPs; immune markers). Maternal infection with *T. gondii* and CMV was associated with a change in APPs among controls but not among individuals who later develop non-affective psychosis. Furthermore, interaction between maternal infection and low levels of APP in the development of psychotic disorder was indicated.

In Study III, the association between hospital admission for infection during childhood (0-13 years of age) and the risk of developing non-affective psychosis was examined. Additionally, possible vulnerable ages, and type of infection (virus and bacteria as well as infection of the central nervous system (CNS)) were scrutinized. Any infection during childhood was modestly associated with non-affective psychosis later in life. The strongest estimates were found for bacterial infection and infection of the CNS during pre-adolescence (10-13 years of age).

In Study IV, the association between maternal infection during pregnancy and non-affective psychosis was investigated. We found no evidence of such association. However, maternal infection during pregnancy and maternal psychiatric disorder interacted in the development of psychosis among offspring. Additionally, associations between maternal infection during pregnancy and offspring childhood infection were investigated. Maternal infection increased the relative risk of offspring childhood infection, two factors that interacted in the development of non-affective psychosis.

In conclusion, there were no strong associations between infection during foetal life or childhood and non-affective psychosis overall. However, neonates with mothers exposed to *T. gondii* and CMV infection had substantial increased relative risks of developing psychosis, especially in conjunction with an altered immune response. The interactions between infection during foetal life and genetic vulnerability, neonatal immune alterations as well as with childhood infections in the development of non-affective psychosis indicate that maternal infection during pregnancy does play an important role in the aetiology.

LIST OF SCIENTIFIC PAPERS

I. Blomström Å, Karlsson H, Wicks S, Yang, S, Yolken, RH, Dalman C. Maternal antibodies to infectious agents and risk for non-affective psychoses in the offspring-a matched case-control study. Schizophr Res. 2012 Sep;140(1-3):25-30.

II. Blomström Å, Gardner RM, Dalman C, Yolken RH, Karlsson H. Influence of maternal infections on neonatal acute phase proteins and their interaction in the development of non-affective psychosis. Transl Psychiatry. 2015 Feb 3;5:e502. doi: 10.1038/tp.2014.142.

III. Blomström Å, Karlsson H, Svensson A, Frisell T, Lee BK, Dal H, Magnusson C, Dalman C. Hospital admission with infection during childhood and risk for psychotic illness--a population-based cohort study. Schizophr Bull. 2014 Nov;40(6):1518-25.

IV. Blomström Å, Karlsson H, Gardner RM, Jörgensen L, Magnusson C, Dalman C. Associations between maternal infection during pregnancy, childhood infection and the risk of subsequent psychotic disorder – a Swedish cohort of nearly 2 million individuals. [Manuscript]

CONTENTS

LIST OF ABBREVIATIONS

APP	Acute phase protein
CI	Confidence interval
CMV	Cytomegalovirus
CNS	Central nervous system
CNV	Copy number variants
GWAS	Genome-wide association studies
HR	Hazard ratio
HSV-1	Herpes simplex virus type 1
HSV-2	Herpes simplex virus type 2
IgG	Immunoglobuline G
IgM	Immunoglobuline M
ITR	Income and Taxation Register
KYNA	Kynurenic acid
LISA	Longitudinal Integration Database for Health Insurance and Labour market studies
MBR	Medical birth register
MGR	Multi-Generation Register
MHC	Major histocompatibility complex
NDBS	Neonatal dried blood spots
NPR	National patient register
OR	Odds ratio
PCA	Principal component analyses
PHC	Population and Housing Census
PVS	Psykiatriskt Vård System
SNP	Single nucleotide polymorphism
T. gondii	*Toxoplasma gondii*
TPR	Total Population Register

1 INTRODUCTION

Non-affective psychoses including schizophrenia are in many cases disabling mental disorders associated with symptoms such as hallucination, delusion and cognitive deficit, which are usually detected and become clinically relevant in early adulthood. In Sweden, psychotic disorders account for approximately 35% of all psychiatric inpatient care [1]. The total cost, including medical care, production loss, and social welfare benefits, is estimated at 25 billion SEK per year [2]. Such disorders constitute a heavy burden for both the affected individual and her/his family members, due to lifelong medical treatment, stigmatization, and alienation from society .

The aetiology of non-affective psychotic disorders is unknown, but increasing evidence suggest that schizophrenia is a neurodevelopmental disorder [3]. Family studies indicate the presence of a strong genetic factor, but no specific gene(s) have yet been identified [4,5]. In fact, most individuals who develope psychotic disorder do not have relatives with psychosis [6]. It is likely that a combination of genetic vulnerability and one or more environmental factor/-s causes the development of the disorder [7,8], suggestive of a two-hit model. Numerous environmental risk factors present during brain development have been proposed, e.g., perinatal complications [9,10], winter season birth [11], urban upbringing [12], and infection [13,14].

The observed excess of winter births among individuals who later develop psychosis and the growing interest in the theory of disturbed neurodevelopment in the aetiology of psychosis has led to the hypothesis of infection during brain development as a risk factor for psychotic disorder [15]. It is well known that certain congenital infections (e.g., rubella virus) can disturb neurodevelopmental processes and cause persistent sequelae ranging from mild to severe [16]. Despite the fact that studies with an ecological design, small studies based on individual data or biological samples as well as animal experimental studies collectively indicate an association between exposure to an infection early in life and adult psychosis, the results are inconclusive and further research is nessesary [13,14,17,18]. Moreover, brain development is not confined to the foetal period but continues throughout childhood and early adulthood [19]. Nevertheless, studies investigating the association between infection during these years on the subsequent development of psychotic disorder are sparse and their results ambiguous [14].

The overall aim of the present thesis is to scrutinize the role of infection during foetal life and childhood in relation to the risk of developing non-affective psychosis, by combining population based registers and individual biological samples. The thesis will hopefully contribute to the understanding of the risk of exposure to infection early in life in relation to psychotic disorder.

Figure 1 illustrates infection during foetal life and childhood in relation to the development of psychotic disorder.

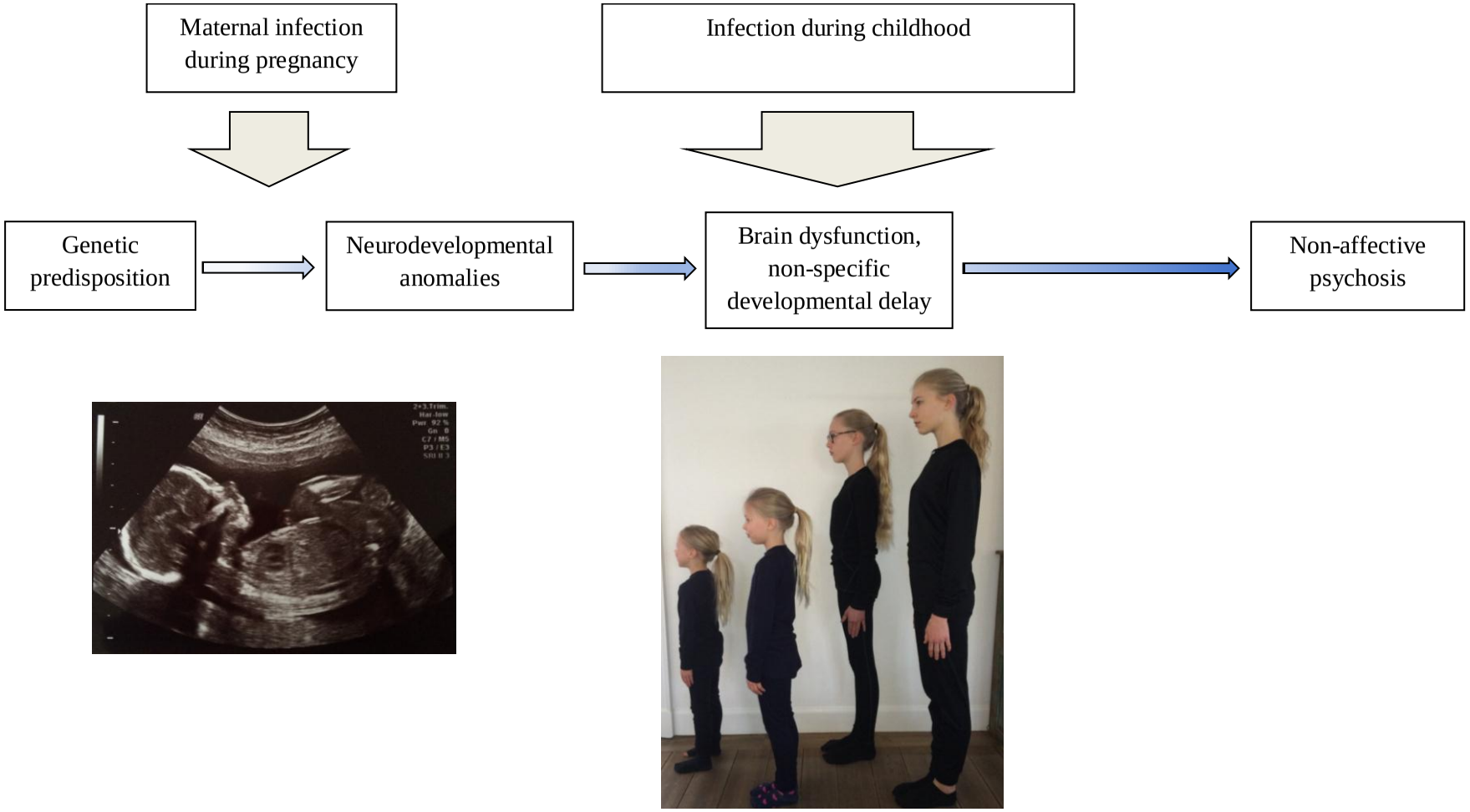

Figure 1. Conceptual model of infections during foetal life and childhood as risk factors for non-affective psychosis.

2 BACKGROUND

2.1 NON-AFFECTIVE PSYCHOSIS INCLUDING SCHIZOPHRENIA

2.1.1 Symptoms and diagnostic criteria

Schizophrenia is the most common syndrome among the large group of non-affective psychoses, accounting for about 50% of cases [20]. For a diagnosis of schizophrenia there must be both psychotic symptoms of a disturbed perception of reality and a social or occupational loss of functioning for a certain period of time. The diagnosis is thus based on symptoms, function and progression. Individuals diagnosed with schizophrenia exhibit positive symptoms such as: hallucinations, delusions, thought disorder, and disorganized or catatonic behaviour, as well as negative symptoms such as: apathy, monotonic speech, and flat facial expressions and with more or less cognitive deficit [21]. Other syndromes within the large group of non-affective psychoses have similar symptoms but some may be more accentuated, or of shorter duration.

There are two diagnostic manuals used by psychiatric clinicians and researchers in many parts of the world: the Diagnostic and Statistical Manual of Mental Disorders (DSM) developed by the American Psychiatric Association and the WHO International Classification of Diseases (ICD). The specific criteria in ICD-10 for a diagnosis of schizophrenia can be found below. The various editions of the two manuals have been gradually coordinated, but there is one significant difference between the two systems: according to the DSM-IV, the requirement for a diagnosis is one month of specific symptoms and at least six months of functional loss, while in the ICD-10 the symptoms must be present for at least one month before the diagnosis can be made, thus the criteria is somewhat broader than in the DSM-IV. In Sweden, the health care registration system only allows ICD codes.

The disorder typically develops gradually during a prodromal phase in late adolescence with a psychotic episode resulting in admission and a diagnosis a few years later [22]. Usually men develop the disorder earlier than women with a mean age of 28.2 years at first admission, whereas women have a mean age of 32.2 years at first admission [23]. In addition, men appear to have a more serious disease course including more cognitive dysfunctions, alcohol and substance abuse [24].

In this thesis the terms non-affective psychosis, psychotic disorder and psychosis will be used interchangeably. However, the terms always refer to non-affective psychosis. Thus, affective psychoses are not included.

ICD-10 diagnostic criteria for schizophrenia

F20 SCHIZOPHRENIA (ICD-10 criteria [25])

G1. Either at least one of the syndromes, symptoms and signs listed below under (1), or at least two of the symptoms and signs listed under (2), should be present for most of the time during an episode of psychotic illness lasting for at least one month (or at some time during most of the days).

1. At least one of the following:
   a. Thought echo, thought insertion or withdrawal, or thought broadcasting.
   b. Delusions of control, influence or passivity, clearly referred to body or limb movements or specific thoughts, actions, or sensations; delusional perception.
   c. Hallucinatory voices giving a running commentary on the patient's behaviour, or discussing him between themselves, or other types of hallucinatory voices coming from some part of the body.
   d. Persistent delusions of other kinds that are culturally inappropriate and completely impossible (e.g. being able to control the weather, or being in communication with aliens from another world).
2. or at least two of the following:
   e. Persistent hallucinations in any modality, when occurring every day for at least one month, when accompanied by delusions (which may be fleeting or half-formed) without clear affective content, or when accompanied by persistent over-valued ideas.
   f. Neologisms, breaks or interpolations in the train of thought, resulting in incoherence or irrelevant speech.
   g. Catatonic behaviour, such as excitement, posturing or waxy flexibility, negativism, mutism and stupor.
   h. "Negative" symptoms such as marked apathy, paucity of speech, and blunting or incongruity of emotional responses (it must be clear that these are not due to depression or to neuroleptic medication).

G2. Most commonly used exclusion criteria: If the patient also meets criteria for manic episode (F30) or depressive episode (F32), the criteria listed under G1.1 and G1.2 above must have been met before the disturbance of mood developed.

G3. The disorder is not attributable to organic brain disease (in the sense of F0), or to alcohol- or drug-related intoxication, dependence or withdrawal.

2.1.2 Incidence and prevalence

The incidence of schizophrenia varies across geographic areas, both between and within countries. To date, the largest systematic review of the incidence of schizophrenia includes 55 studies from 33 countries and report a median incidence rate of 15.2 (7.7-43) per 100 000 persons, and with a males-females ratio of 1.4:1 [26]. In general, higher incidence rates are observed in urban areas and among migrants.

While incidence rate refers to the number of new cases of a disease per population at risk per time unit, prevalence is the number of cases in a population at a given time point. In a large systematic review including 142 studies from 46 countries, Saha *et al.*, reported the prevalence to be 4.6 (1.9-10.0) per 1 000 individuals [27]. Less developed countries tended to have lower prevalences than more developed countries.

There are no reports on incidence or prevalence for the whole of Sweden. However, Jörgensen *et al*., recently reported on the incidence and prevalence of schizophrenia and non-affective psychosis, respectively in Stockholm County Council [20,28]. The incidence of schizophrenia was 28 per 100 000 individuals, while in the more broadly defined group of non-affective psychoses, the incidence was 78 per 100 000. The one year prevalence was estimated at 3.7 per 1 000 individuals for schizophrenia, and 6.7 per 1 000 individuals for the larger group of non-affective psychoses.

2.1.3 Neurodevelopmental hypothesis

When Kraepelin first described schizophrenia over a hundred years ago, he used the term Dementia praecox, indicating a degenerative process [29]. Schizophrenia was long considered a neurodegenerative disorder like Alzheimer´s disease. However, in 1987 the neurodevelopmental hypothesis was presented [30,31]. Although the hypothesis of a progressive degenerative course has not been disregarded [32,33], evidence of disturbed neurodevelopment in schizophrenia has been amassing [34]: brain morphological changes present before onset and not correlating with disease duration are reported [35-37]. In contrast, gliosis, which is normally seen in degenerative brain disorders, is not present [38,39]. Moreover, prenatal and early childhood complications such as obstetric complications, delayed motor development, and lower cognitive abilities are more common among individuals who develop schizophrenia [10,40-43]. In addition, genetic studies (GWAS) have reported variations in genes involved in brain development [5]. More recently, it has been suggested that the two theories of neurodevelopmental insults and degeneration are not to be mutually exclusive but linked with each other [44,45].

2.1.4 Genetic risk and environmental influences

At an individual level, the strongest risk factor for developing schizophrenia is having a first degree relative with the disorder [46]. However, most individuals who develop schizophrenia do not have any family history of the disorder. No causal gene has so far been identified. However, GWAS have found common genomic variations such as single nucleotide polymorphisms (SNPs) [5] as well as rare variations, e.g., copy number variations (CNVs) [4], to be associated with schizophrenia. A SNP is a DNA-site anywhere on the genome where one single nucleotide base pair varies between individuals. Currently over 100 common SNPs have been associated with schizophrenia and appear to cluster in genes involved in CNS functions [47]. Some of the strongest associations have been observed in the major histocompatibility complex (MHC) region on chromosome 6. This region is dense with genes associated with the immune system but also genes that are involved in brain development and plasticity [48,49]. CNVs are rare structural variations that include a large number of base pairs ranging from thousands to millions that have been either deleted, or duplicated. These can be situated inside or outside genes and may be inherited or appear *de novo*. Patients with schizophrenia appear to harbour more CNVs than controls, some of which are situated within genes important for brain development and immunity [4,50,51].

Among affected monozygotic twins, both twins have the disorder in 40-60% of cases [52], which indicates a substantial contribution of environmental effects. It is likely that genetic factors and one or more environmental factors are needed to develop the disease. Interactions between genetic and environmental factors such as infection have in fact been suggested in schizophrenia aetiology [8].

2.2 INFECTIONS EARLY IN LIFE – POTENTIAL RISK FACTORS FOR NON-AFFECTIVE PSYCHOSIS

The theory of neurodevelopmental insults in schizophrenia aetiology, the increasing knowledge of various pathogens causing congenital brain anomalies, the awareness of psychotic symptoms during certain infections and birth excess during the winter months (when viral infections are highly prevalent) among children who later develope schizophrenia increased the interest in prenatal exposure to viral infection in the aetiology of schizophrenia in the 1970s [53]. In 1988, Mednick *et al*., presented their seminal study of the increased incidence of schizophrenia among children born in Finland during the influenza epidemic in 1957 [15]. Since then, maternal Influenza A infection during pregnancy has been the topic of numerous studies. Most of these studies have used an ecological design without information about exposure at an individual level and the results have been contradictory [54].

After the idea of prenatal infection in the aetiology of psychotic disorder was presented, several studies on this association were conducted using *individual* data. Some investigated biological samples (sera and blood), while others examined clinical diagnoses. A summary of these studies is presented below.

It is important to note that in the context of investigating maternal infection during pregnancy in schizophrenia research terms, such as “prenatal infection”, “foetal exposure to infection”, or “infection during foetal life” are often used. However, these terms actually refer to *maternal* infection during pregnancy. Whether the foetus is infected is not known. In this thesis these terms will be used interchangeably, but always refer to maternal infection during pregnancy.

2.2.1.1 Studies using biological samples

Basically, there are three ways of detecting infection in sera or blood: by direct detection which can be done by culture (a certain amount of blood is needed), by detecting microbial genomes using the PCR-technique or by testing for the presence of antibodies specifically directed at a certain microbe (serology). This latter approach has the advantage of being sensitive, cheap and capable of detecting exposures long after the microbe is cleared by the host immune system.

Serology determines antigen-specific immunity in terms of levels of antibodies directed at the specific pathogen. Antibodies are part of the adaptive immune response, which is a specific defense against a certain pathogen. Immunoglobulin M (IgM) antibodies are the first antibodies to be produced within 1-2 weeks after exposure. They are only detectable for a few

weeks and gradually replaced by immunoglobulin G (IgG) antibodies. IgM detection therefore indicates an ongoing or recent infection. IgG antibodies are produced after a couple of weeks and can usually be detected for years after exposure. Thus, IgG detection reveals whether or not an individual has been exposed but does not provide information about when the exposure occurred. Neonatal blood harbours levels of IgG mainly derived from the mother and transferred across the placenta during pregnancy to provide the newborn with passive immunization [55]. Thus, IgG in maternal sera and in neonatal blood mirrors maternal exposure to an infection at some time prior to testing/delivery.

A few serological studies using stored maternal sera or archived dried neonatal blood samples have investigated the association between maternal infection and future psychosis development among the offspring [56-64] (Table 1).

Table 1. Serologic studies on the association between maternal levels of IgG directed at *T. gondii*, CMV, HSV-1, and -2, Rubella, and Influenza and psychotic disorder among offspring.

Samples	Published studies	Case definition	Type of sample	Association with psychotic disorder
National Collaborative Perinatal Project (NCPP), USA Born 1959-1966	Buka *et al*., 2001	"Major psychotic disorder", n=27	Stored maternal sera	*T. gondii: t* test=0.51 , p=0.61 CMV: *t* test=-0.42, p=0.68 HSV-1: *t* test=-0.57 , p=0.57 **HSV-2: *t* test=2.11, p=0.04**
	2008	n=108		**HSV-2: OR 1.8, 95% CI: 1.1-3.0**
	Xiao et al., 2009	n=120		*T. gondii*: OR 1.3, 95% CI: 0.6-2.8
The Prenatal Determinants of Schizophrenia Study (PDS), USA Born 1959-67	Brown *et al*., 2005	"Schizophrenia spectrum disorder", n=63	-"-	***T. gondii*: OR 2.6, 95% CI: 1.0-6.8**
	2006	n=60		CMV: OR 1.1, 95% CI: 0.5-2.4 HSV-1: OR 1.0, 95% CI: 0.5-2.1 HSV-2: OR 1.1, 95% CI: 0.5-2.6
Danish birth cohort, Born since 1981	Mortensen *et al*., 2007	Schizophrenia, n=186	Archived dried neonatal blood samples	***T. gondii*: OR 1.8, 95% CI: 1.0-3.2** HSV-1 and -2: No significant association, data not shown
	2010	n=602		**HSV-2: OR 1.4, 95% CI: 1.0-1.9**
Swedish birth cohort Born 1975-85	Blomström *et al*., 2012	Schizophrenia, n=47	-"-	*T. gondii*: OR 2.0, 95% CI: 0.9-4.7 CMV: OR 2.1, 95% CI: 0.9-4.9 HSV-1: OR 0.8, 95% CI: 0.4-1.6 HSV-2: OR 0.5, 95% CI: 0.2-1.4

All the studies are based on three collections, two American collections of maternal sera from pregnant women in the 1960s, and one sample based on dried blood from neonates born in Denmark in the 1980s. The focus has been on infections with *Toxoplasma gondii*, cytomegalovirus, herpes simplex virus types 1 and 2 as well as rubella virus (i.e., TORCH-

infections) during pregnancy, all of which are known to cause congenital malformations in the offspring [65-67].

There are several methodological limitations in these studies. Half of the studies had fairly small sample sizes and few exposed cases [56,59-62]. Moreover, some of the studies used the same participants when reporting on exposure to individual pathogens [57,58,60,61]. It is possible that these groups of mothers were exposed to several infections due to related factors such as genetic susceptibility and life-style. However, inclusion of such potential confounders was limited. Moreover, in serologic studies there is a risk of exposure misclassification when exposure status has to be defined in terms of cut-off levels of antibodies. Although information on the prevalence of the infectious diseases in the time period and geographic area in question would be important, some of the studies from the US did not contain such information. Another shortcoming that could complicate comparisons between the studies is that psychotic disorders are sometimes included as a mixed group, and sometimes as the more restricted diagnosis of schizophrenia. Moreover, the Danish studies had a short follow-up duration whereby the finding can only be generalized to early onset schizophrenia [63,64]. Overall, the results are contradictory and have limited generalizability.

In Sweden, rubella vaccination has been part of the national vaccination programme since 1974 [68] and apart from isolated outbreaks in specific regions, rubella is very rare in Sweden [69]. The methods of detecting the highly variable antigenic structure of the different *Influenza* virus strains common between 1975 and 1985 require more blood than eligible from NDBS samples. Therefore, the present thesis focused on *Toxoplasma gondii*, cytomegalovirus, and Herpes simplex virus types 1 and 2. Brief information about these pathogens can be found below.

Toxoplasma gondii

Toxoplasma gondii (*T. gondii*) is a parasite found in, for example, undercooked meat, poorly rinsed vegetables, contaminated water and cat litter. The parasite has a complicated life cycle and requires a feline host for the reproductive part of the cycle. Oocysts are excreted in feline faeces, whereby humans (or other warm blooded animals) may be infected by the aerosolized oocysts as a result of, for example, playing in sandpits, cleaning cat litter trays or ingesting the cysts (the latent stage) in undercooked meat. The parasite establishes a lifelong latent infection in a slowly replicating bradyzoite stage, hiding inside cysts in cells of the intermediate host [70]. However, in case of immune suppression, e.g., AIDS, it may become active again by entering the rapidly replicating tachyzoite stage [71]. The parasite is distributed worldwide with a seroprevalence in the human population of about 30%, although there is a variation of between 10-80% [72]. In Sweden, the seroprevalence is 15-25% among pregnant women, decreasing with latitude [73]. Globally, the incidence rate of congenital toxoplasmosis is estimated at 1.5 cases per 1 000 live births [74]. Congenital toxoplasmosis is very rare in Sweden, with a prevalence of 0.73/10 000 births [73].

The parasite has a preference for the foetal brain and primary infection with *T. gondii* during pregnancy is associated with brain malformation such as microcephaly, enlarged ventricles and blindness [75,76]. However, also asymptomatic congenitally infected children may develop sequelae such as cognitive and motor impairments similar to those found in individuals with schizophrenia [77].

Cytomegalovirus

Cytomegalovirus (CMV) is transmitted through body fluids such as saliva, blood, breast milk, urine and semen. CMV occurs worldwide but the prevalence varies and in developing countries it can be as high as 100% among pregnant women [78]. In the US the prevalence is around 50% [79], while in Sweden, the prevalence is around 80% in the adult population [80]. Individuals with CMV have a lifelong latent infection (inactive chronic infection) and may become re-infected by other strains [81]. Similar to *T. gondii* the chronic infection may re-activate upon immune deficiency, such as at transplantation, or HIV infection [82]. Among mothers with a chronic infection (preconceptional immunity), transmission of the virus may take place from the mother to the foetus either from a primary infection with a new strain of the virus, or from reactivation of the chronic infection [83,84]. The prevalence of congenital infection in Europe is low, around 0.2-0.5% [85], and about 60% of the infected neonates have been infected by mothers with chronic infection [86]. In about half of all cases of transmission to the foetus, maternal primary infection causes clinical symptoms such as brain malformation and intrauterine growth restriction, as well as neurodevelopmental sequelae, e.g., sensorineural hearing loss, mental retardation and motor impairment [87]. MRI brain scans of children with congenital CMV infection show decreased brain volume in the temporal lobe and hippocampus, which resembles that seen in schizophrenia patients [88,89]. Neonates born to mothers with a chronic infection present these symptoms less frequently. However, an asymptomatic congenital CMV infection can lead to more subtle cognitive deficits later in childhood [90].

Herpes simplex virus type 1 and -2

Herpes simplex virus type 1 and 2 (HSV-1 and -2) can be sexually transmitted, as well as transmitted from body fluids such as saliva. In general, type 1 is the labial variant, and type 2 the genital variant, but both can occur in either location. After the acute stage, these viruses establish latent infections in sensory nerve ganglia [91]. The prevalence has increased worldwide in recent decades and apart from Asia where it is somewhat lower and Africa where it is higher, the global prevalence in the adult population is similar to Swedish figures: HSV-1 60% and HSV-2 25% [92,93]. Although transmission from mother to neonate usually occurs during delivery [94], about 5% of neonates are infected during foetal life [95]. Apart from more common skin and mucus lesions, prenatal infection with Herpes simplex virus can cause CNS infection with microcephaly and hydranencephaly [96]. In particular, infection with HSV-2 is associated with mortality and long-term sequelae such as mental retardation and cognitive impairments [97,98].

2.2.1.2 Studies with individual clinical diagnoses

In addition to studies using sera or blood, register based studies with individual clinical diagnoses have explored various groups of maternal infections during pregnancy in relation to psychotic disorder in the offspring [99-103] (Table 2).

Similar to the serological studies, most of these register based studies had methodological limitations. The studies from the US and Finland had small sample sizes, and few exposed cases [99-101] an some contained a risk of exposure misclassification [99,102,103]. In general, all studies had a rather short follow-up duration and limitations in the ability to control for confounding factors. Overall, associations with respiratory infections, pyelonephritis (boarder line significant) and bacterial infection were reported, thus indicating specific effects of bacterial infections [99,101,102]. However, the topic requires further investigation.

Table 2. Register based studies on the association between maternal infection during pregnancy and psychotic disorder among offspring.

Samples	Published studies	Exposure	Type of psychotic disorder	Association with psychotic disorder
The Prenatal Determinants of Schizophrenia Study (PDS), US cohort Born 1959-67 N=7 800	Brown *et al*., 2000	Respiratory infection	SSD*, n=58	**OR 2.1, 95% CI: 1.0-4.4**
	Babulas *et al*., 2006	Genital/reproductive infection (1st trimester)	SSD*, n=71	RR 0.9, 95% CI: 0.2-3.7
Finnish national cohort Born 1947-90 N=23 400	Clarke *et al*., 2009	Pyelonephritis	Schizophrenia, n=71	OR 1.5, 95% CI: 0.9-2.4
Danish cohort Born 1959-61 N=7 900	Sorensen *et al*., 2009	Viral infection	Schizophrenia, n=153	OR 0.5, 95% CI: 0.1-2.1
		Bacterial infection		**OR 2.1, 95% CI: 1.1-4.3**
Danish national cohort Born 1978-98 N=1 115 700	Nielsen *et al*., 2013	Any infection	Schizophrenia, n=3 700	**RR 1.2, 95% CI: 1.0-1.4**

*Schizophrenia spectrum disorder

Interestingly, both Clarke *et al*., and Nielsen *et al*., found synergism between maternal psychotic disorder and maternal infection during pregnancy in relation to the risk of schizophrenia development in the child [101,103]. These findings support the theory of gene-environment interaction in the development of schizophrenia [8].

Nielsen *et al*., investigated maternal hospital admission with infection before, during and after pregnancy as one variable and found that it had an equally strong association with schizophrenia in the offspring as infection during pregnancy [103]. In addition, paternal infection before, during and after pregnancy was weakly associated with offspring schizophrenia. The authors suggest that a common factor increases the propensity for infection and schizophrenia separately rather than a causal effect of the infection *per se*. However, as infection after pregnancy was included the infection may very well have been

transmitted to the child [104,105]. Thus, these results do not exclude a specific effect on the foetus due to infection during pregnancy.

Larger studies with a sufficiently long follow-up to include the majority of cases of schizophrenia and assessing them in relation to specific infections, timing of infection and interaction with genetic susceptibility to schizophrenia is desirable in order to determine underlying biological mechanisms [13].

2.2.2 Infection during childhood

The theory of exposure to infection affecting the brain during development has subsequently evolved to include not only exposure in prenatal life but also during childhood. Brain development continues throughout childhood, making it another period that is sensitive to disruption [19]. Until recently, associations between childhood infections and psychotic disorder had only been investigated in terms of infections in the central nervous system (CNS) [106-112] (Table 3).

Table 3. Cohort studies on the association between clinical diagnosis of CNS infection during childhood and psychotic disorder.

Samples	Published studies	Exposure	Type of psychotic disorder ()=exp	Association with psychotic disorder	
				Virus	Bacteria
Finland, 1966-80 N=11 017	Rantakallio *et al*., 1997	CNS infection ≤14 years	Schizophrenia, n=76 (4)	**OR 4.8, 95% CI: 1.6-14.0**	
	Koponen *et al*., 2004		Other psychoses, n=53 (2)		**OR=6.8, 95% CI 1.4-32.8**
			Schizophrenia, n=100 (4)	OR 2.5, 95% CI: 0.9-7.0	
Britain, 1958 N=17 414	Leask *et al*., 2002	"Common childhood illness" Meningitis ≤12 y	"Narrow schizophrenia" n=35 (1)	**OR= 7.8, 95% CI 1.0-59.0**	
Finland, 1960-91 N=320	Suvasaari *et al*., 2003	CNS infection ≤14 years	Schizophrenia, n=3 (exp)	CI=0.94% (Cumulative Incidence)	
Brazil, 1971-74 N=346	Abrahao *et al*., 2005 sibling controls	Meningococcal meningitis ≤4 years	Schizophrenia n=8 (8) Other psychosis n=25 (21)		**4.2% vs 0%** **12.1% vs 2.8%**
Sweden, 1973-85 N=1 187 553	Dalman *et al*., 2008	CNS infection ≤12 years	Non-affective psychosis n=2 269 (23)	RR=1.3, 95% CI: 0.8-2.0	RR=0.9, 95% CI: 0.3-2.4
			Schizophrenia (8)	**RR=1.6 95% CI: 1.0-2.5**	RR=0.9 95% CI 0.4-2.5
Israel, 1970-87 N=9 970	Weiser *et al*., 2010 Gastroenteritis controls	CNS infection ≤15 years	Schizophrenia n=72 (24)	HR=0.7 95% CI: 0.4-1.2	HR=1.7 95% CI: 0.7-4.1

Most of these studies have methodological limitations. For example, four of the studies had between one and four exposed cases [106-109], while Weiser *et al*., used controls with another type of infection, which may have diluted the results [112]. Abrahao *et al*., had a huge loss to follow-up of 96% [110]. All studies also had a short-term follow up.

In a meta-analyses conducted by Khandaker *et al*., only two of the studies presented in Table 3 were included in the analyses due to methodological limitations [107,111]. A significant association between childhood CNS infection and non-affective psychosis was reported, with a pooled risk ratio of 1.6. Differentiation between bacteria and virus indicated that the association was driven by viral CNS infections. However, the two studies included had a relatively short follow-up duration and small sample sizes.

Recently, infections other than CNS before the age of three years were reported to increase the risk of schizophrenia in a male cohort from Australia [113]. Considering these observations, *any* infection during childhood, CNS or non-CNS, could imply a risk of psychotic disorder, but has not been sufficiently investigated.

Altogether, no large studies with a long-term follow-up, that investigate the effect of *any* as well as specific infections during childhood have been made. In addition, a temporal association between childhood infection and psychotic disorder is lacking.

2.2.3 The "two-hit" model

The "two-hit" model hypothesizes a genetic "first hit" with mutations in schizophrenia candidate genes involved in brain development and a "second hit" further affecting the function of these genes, which subsequently leads to the development of psychotic disorder [114]. A number of stressors including infection has been suggested, but more research is necessary to confirm the hypothesis [115]. In agreement with the two-hit model, an insult on the developing brain during foetal life in terms of immune activation or infection could induce an increased vulnerability to a second hit [116]. Immune alterations have in fact been observed in individuals who subsequently develop schizophrenia [117,118]. In addition, maternal infection during pregnancy may alter the infants immune response, an effect of which can increase the susceptibility and vulnerability to infection for years [119]. Despite these facts, no study to date has examined interaction between maternal infection during pregnancy and infection during childhood in the risk of developing non-affective psychosis.

2.2.4 Infection during foetal life and childhood – what does it implicate?

Exposure to infection can involve risks due to common mechanisms and not the pathogen *per se*. Hypotheses on the underlying factors associated with infection are presented below.

2.2.4.1 Maternal immune activation (MIA) and inflammation

Immune activation including inflammation is part of the host response to infection, by which cellular and humoral mechanisms attempt to contain and eliminate the pathogen.

In 1997 Gilmore and Jarskog proposed that pro-inflammatory cytokines might be involved in the causal pathway of psychosis development [120]. Importantly, cytokines can pass over the placenta, and cross the blood-brain barrier [121-123]. However, so far the clinical support for an association between maternal immune activation (MIA) and schizophrenia development in the offspring is fairly weak. In terms of cytokines as a risk factor, two case-control studies, one using maternal second trimester sera and the other third trimester sera, found associations with IL- 8 and TNF-α, respectively, but not with other cytokines, and were unable to replicate each other's results [124,125]. More recently, Nielsen et al., analysed a number of inflammatory markers, including the aforementioned, from neonatal blood and found no associations [126]. Two other studies support the role of MIA, due to findings that neonates of mothers with increased levels of activated C1q (the initiator molecule of the complement cascade) respectively the APP CRP have a higher risk of developing psychotic disorder [127,128]. In contrast, a large number of experimental studies in pregnant rodents support the role of MIA in the development of psychosis-related behaviours [17,129].

There are over seventy cytokines involved in cell-signaling with a broad range of specific and overlapping functions. The action of cytokines is highly dependent on specific receptors located on the cell itself (autocrine signaling), neighbouring cells (paracrine signalling), or distant cells reached via the circulation (endocrine signalling) [130]. Consequently, it is difficult to fully understand and interpret results from snapshot analyses of cytokine levels. However, given that MIA is an important player in psychosis development, associations between any severe infections during pregnancy and non-affective psychosis should be observed.

2.2.4.2 Deficient immune response

Another interpretation of the association between schizophrenia and various infections concerns a deficient immune response [131,132]. In fact, patients with schizophrenia suffer more frequent infections compared to controls [133,134]. More importantly, infection as a cause of death is much higher among patients with schizophrenia than the general population [135-137], indicating a vulnerability to such exposure. However, these associations may be biased by life-style factors (e.g., obesity, drugs) and delayed healthcare contact [138]. There are two studies that provide clinical support for a deficient innate immune response among individuals with psychotic disorder. In an experimental study Müller *et al.*, demonstrated that monocytes (white blood cells) obtained from schizophrenia patients had a decreased in vitro response to viral and bacterial components compared to such cells obtained from controls [118]. Gardner *et al*., measured levels of acute phase proteins (APP) in neonatal blood and found that neonates who later develop non-affective psychosis had lower levels of some APPs compared to controls [117]. APPs are serum proteins that form part of the innate immune response (the first line defense). Upon infection/inflammation their release is triggered by cytokines. They are involved in many steps of the inflammatory response, as well as in coagulation and transport. Similar to cytokines, their regulation is complex with an additive, inhibitory, and synergistic effect on each other [139]. However, in contrast to cytokines, these

proteins fluctuate more slowly and can remain elevated for long periods of time following an infection. Hence, altered levels of APPs are much more likely to be captured.

Relevant to this thesis is the fact that both cytokines and acute phase proteins are peripherally acting immune proteins involved in protection against infection as well as in the cross-talk between the immune system and the CNS. In addition, they are involved in the formation and plasticity of the developing brain [122,140]. Thus, they could be part of the pathway between infection and psychotic disorder [141]. In this regard, altered immunity in the aetiology of schizophrenia has been proposed [131]. Nevertheless, interaction between maternal infection during pregnancy and neonatal immune activity in the development of psychotic disorder has not been investigated.

2.2.4.3 Affected microbiom - antibiotics

It is well known that there is communication between the microbiom, the gut, and the brain [142]. It is also becoming increasingly evident that the composition of the microbes colonizing the gut plays an important role in brain function and brain development as reviewed in [143], while it has also been suggested that the microbiom plays a role in the aetiology of neurodevelopmental disorders [144]. Indeed, Heijitz *et al*., demonstrated in a mouse model that the microbiom affects the level of DA in the brain [145]; mice with a sterile gut (without a "normal" microbiom) had an elevated DA and behaved hyperactively compared to mice with "normal" gut flora. Presumably, all individuals with severe bacterial infections (e.g., treated in hospital) receive antibiotics, which inevitably alter the microbiom in the gut to a greater or lesser degree and may thus have implications for neurodevelopmental disorders such as schizophrenia. Only one study has reported bacterial infection during pregnancy to be associated with schizophrenia in the offspring. No study has investigated bacterial infection during childhood other than in the CNS and risk of psychotic disorder. This subject needs further attention.

2.2.4.4 Genetic susceptibility

As mentioned previously, GWAS have identified common genetic variants (SNPs) associated with schizophrenia. Some of these are located within genes that are also connected with infection. In particular, common variants in a region within a large gene on chromosome 6, which encode the major histocompatibility complex (MHC), have been reported to be associated with schizophrenia [5,146]. MHC is a molecule responsible for anti-gene presentation to T lymphocytes and essential in the response to infection. More recently, interactions between SNPs in the MHC region and maternal CMV and HSV-1 infection in schizophrenia risk have been reported [147,148]. Additionally, GWAS has reported on interactions with other genetic variations and maternal exposure to *T. gondii* and CMV [147,149]. Thus, analyses of interactions between genetic susceptibility and infection (both specific infections and infection in general) early in life in the risk of developing psychotic disorder need further investigation.

2.3 SUMMARY

Numerous studies have addressed the effect of infection during foetal life and childhood on the risk of developing psychotic disorder. However, contradictory findings, methodological limitations, and knowledge gaps highlight the need for further attention on the subject. In order to increase consistency and disentangle the mechanisms by which early infection may be involved in the development of psychotic disorder, it is essential to:

- Conduct large studies with a sufficiently long follow-up to include the peak incidence of schizophrenia.
- Have good control of factors that may confound the association between early infection and psychosis.
- Scrutinize the association with *any* infection and with individual infections during both foetal life and childhood.
- Investigate the relation between maternal infection during pregnancy and neonatal immune responses and subsequent psychosis development.
- Disentangle whether there is susceptibility for infection and for psychosis separately or rather a specific effect by the infection *per se*.
- Explore the risks of multiple hits of infection.

3 AIMS OF THE THESIS

The overall aim of this thesis was to investigate the association between infection during fetal life and childhood and non-affective psychosis later in life.
For that purpose, four studies were designed using health care registers and biological samples, addressing the following research questions:

Study I Is maternal chronic infection with the neurotropic pathogens *Toxoplasma gondii*, cytomegalovirus, herpes simplex virus type-1, and -2 associated with schizophrenia or other non-affective psychoses in the offspring?

Study II Does maternal chronic infection with *Toxoplasma gondii*, cytomegalovirus, herpes simplex virus type-1, and -2 during pregnancy influence the immune activity in terms of levels of acute phase proteins in the neonate? Is the psychosis risk associated with maternal chronic infection dependent of the immune response in the neonate?

Study III Are childhood infections associated with non-affective psychosis later in life? Is the risk associated with all types of infection, or rather specific for certain groups of pathogens (bacteria or virus)? Is the timing and the number of infections of importance?

Study IV Is maternal infection during pregnancy associated with non-affective psychosis in the offspring? Are parental psychiatric disorder (as a marker of genetic vulnerability) and parental infections prior to or during pregnancy acting synergistically in the development of psychosis among the offspring? Is maternal infection during pregnancy associated with infections during childhood, and do their combined effect increase the risk of developing non-affective psychosis (risk of multiple hits)?

4 MATERIAL AND METHODS

Figure 2 illustrates the research questions and the data sources, and the relationship between the four studies included in the thesis.

4.1 REGISTERS AND BIO-BANKS

The studies in the thesis are based on linkages to several registers held by Statistics Sweden and the National Board of Health and Welfare.

The National Patient Register (NPR) includes virtually all psychiatric inpatient care in Sweden since 1973, and outpatient care since 2005 [150]. Somatic inpatient care is completely covered from 1987, but most county councils are included since 1978. The NPR was used to find data regarding any hospital admission including the diagnoses of infection or non-affective psychoses.

The Medical Birth Register (MBR) was initiated in 1973 and includes data from the prenatal, delivery and neonatal periods from about all deliveries in Sweden [151]. Data on gestational age and obstetric complications were retrieved from the MBR.

The Population and Housing Censuses (PHC) were administered every five years between 1960 and 1990 and included, by law, all individuals registered and living in Sweden with information on demographic data [152]. Data on socio-economic status in terms of single household, parental employment, and household receiving social welfare benefits was obtained from the PHC of 1985 and 1990.

The Longitudinal Integration Database for Health Insurance and Labour market studies (LISA) is updated annually since 1990 with a new annual register [152]. The database includes all individuals 16 years of age and older registered in Sweden as of December 31 for each year. The LISA integrates existing data from the labour market, educational and social sectors, and connects family members. Data on socio-economic status in terms of disposable income in the family, and education level among the parents were obtained from the LISA.

The Total Population Register (TPR) was initiated 1968 [152]. This register includes the entire Swedish population and is the basis for all official population statistics. Information such as civil status, place of residence, country of birth, and relations between family members is available. Data on year of migration among parents was retrieved from the TPR.

The Income and Taxation Register (ITR) includes information of income, pensions, and taxes among all individuals registered in Sweden, since 1968 [152]. Data on social welfare benefits was retrieved from this register.

The Multi-Generation Register (MGR) consists of individuals born 1932 or later who have been registered in Sweden at any time since 1961[152]. The register includes information of biological and adoptive parents. Data on biological fathers was obtained from the MGR.

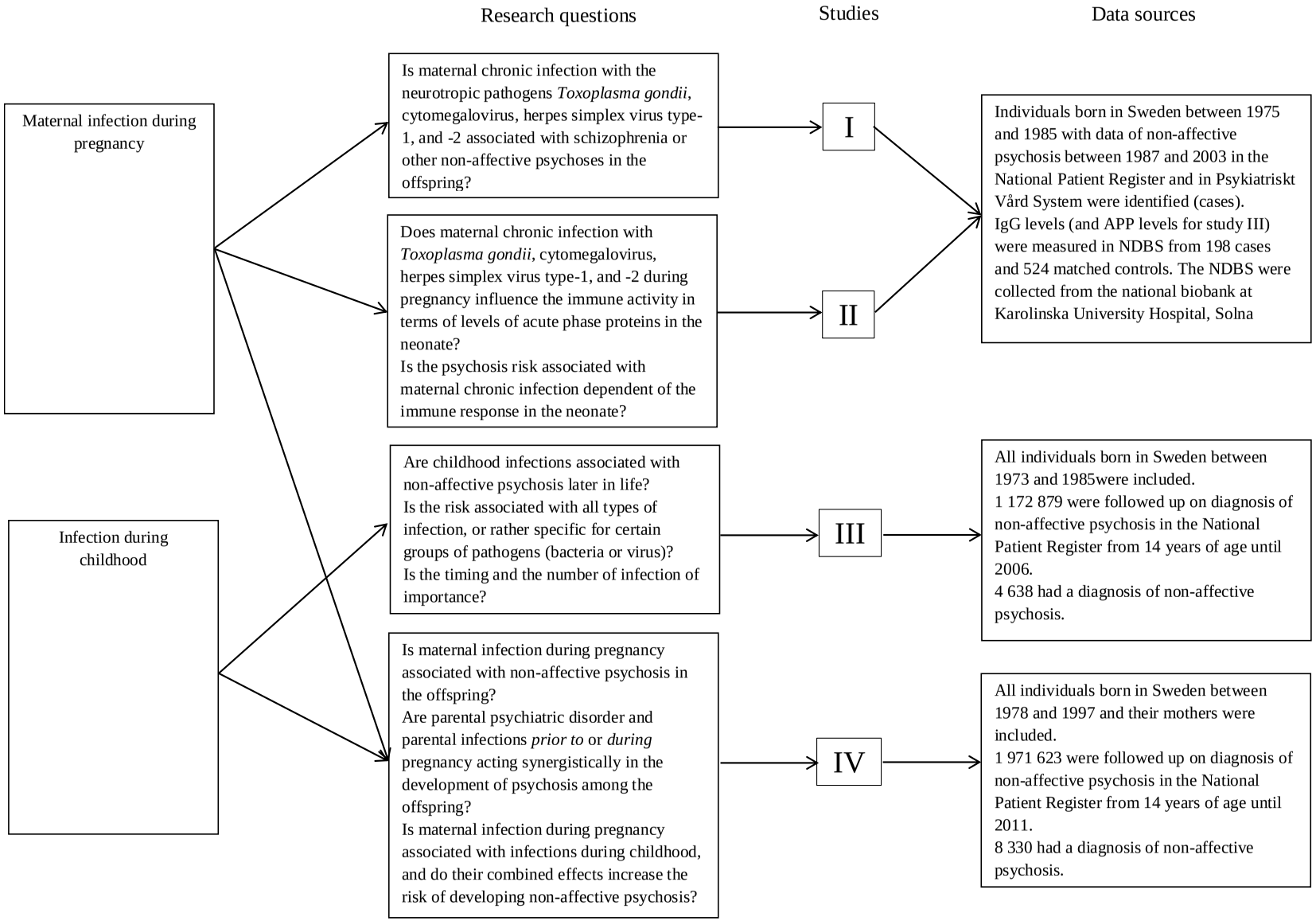

Figure 2. Overview of the research questions, studies and data sources included in the thesis.

Psykiatriskt Vård System (PVS) was a local psychiatric outpatient care register used in Stockholm County Council between 1997 and 2006 [28]. PVS had a 80% coverage and was unique in that it registered diagnoses (both DSM and ICD) and not only visits.

In Sweden, since 1975, blood is collected from all newborns in a screening program for early detection of diseases such as PhenylKetonUria (PKU), and archived on filter paper in a biobank at Karolinska University Hospital, Solna. These neonatal dried blood spots (NDBS) were used for analyzing maternal exposure to infection in terms of antibody levels and neonatal immune reactivity in terms of levels of acute phase proteins (APPs).

4.2 STUDY SAMPLES

Study I and II All individuals born in Sweden between 1975 and 1985 and treated as in- or outpatients in Stockholm County Council until 2004 were included. The participants had to be alive and resident in Sweden on December 31, 2003. Written informed consent was obligate for enrollment in the study.

Study III All individuals born in Sweden between 1973 and 1985, and resident in Sweden at 14 years of age were included in the study and followed up until end of 2006. Adopted individuals and individuals who emigrated before 14 years of age were excluded.

Study IV All individuals born in Sweden between 1978 and 1997, with information on biological father were included and followed up from 14 years of age until end of 2011.

4.3 MEASURES

4.3.1 Exposure

The measure of prenatal and childhood exposure to infections varied in the four studies.

Study I As mentioned previously, serology is an efficient method of determining microbial exposure, and was therefore used in this thesis. Maternal exposure to chronic infection was measured as levels of maternal immunoglobulin G (IgG) antibodies towards the infectious agents *T. gondii*, CMV, HSV-1, and -2 in NDBS. Neonatal blood harbors maternal IgG antibodies predominately derived from the maternal circulation and are transferred during pregnancy, reviewed in [55]. Therefore, neonatal blood explores maternal infection or immune reactivity at some point prior to delivery.

Study II Previously obtained data on maternal exposure to the infectious agents *T. gondii*, CMV, HSV-1, and -2 (See study I) and on neonatal immune activity in terms of levels of APPs measured from the same blood spots [117] were used. APPs are part of the innate immunity and are produced in response to inflammatory cytokines [153]. In general, APPs are not transported across the placenta and thus provide a measure of the activity of the innate immune response in the neonate [154].

Study III Information on primary diagnoses of infection during childhood was retrieved from the NPR. All diagnoses arising from infection was identified in ICD-8, -9 and -10, (Supplementary table S1(Table S1)).

Study IV Information on diagnoses of maternal infection before and during pregnancy, and infection during childhood was retrieved from NPR. As before, all diagnoses of infection was identified in ICD-8, -9 and -10, (Table S1). Mainly the primary diagnosis was retrieved. However, in those cases the primary diagnosis was of obstetric origin the first diagnosis of infection of the seven secondary diagnoses was extracted.

4.3.2 Outcome

Non-affective psychoses were defined according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) and International Classification of Disease (ICD-9 and -10) as follows: DSM-IV: 295.1-.4, .6, .9 Schizophrenia, 295.7 Schizoaffective disorders, 297.1 Persistent delusional disorders, 297.3 Induced delusional disorder, 298.8 Acute and transient psychotic disorders, 298.9 Unspecified nonorganic psychosis and 301.22 Schizotypal disorder; ICD-9 295A-E, G, W and X Schizophrenia, 295F Schizotypal disorder, 297B, C, W and X, Persistent delusional disorders, 297D Induced delusional disorder, 295H Schizoaffective disorders and 298C and E Acute and transient psychotic disorders, 298W Other nonorganic psychotic disorders, 298X Unspecified nonorganic psychosis. Other nonorganic psychoses excluding depressive type psychosis and excitative type psychosis; ICD-10: F20 Schizophrenia, F21 Schizotypal disorder, F22 Persistent delusional disorders, F23 Acute and transient psychotic disorders, F24 Induced delusional disorder, F25 Schizoaffective disorders, F28 Other nonorganic psychotic disorders, F29 Unspecified nonorganic psychosis.

The more narrow diagnosis of schizophrenia was defined as DSM-IV: 295.1-.4, .6, .9 Schizophrenia; ICD-9 295A-D, G, W and X Schizophrenia; ICD-10: F20 Schizophrenia.

4.4 BLOOD SPOT ANALYSES

As part of a neonatal screening program for metabolic diseases, blood from all newborns in Sweden has been collected on filter papers since 1975, and stored at 4°C at a central biobank. Antibodies in filter paper blood spots that have been stored for as much as two decades are stable [155,156]. Half a blood spot from each participant was excised and transferred to a small plastic ziploc bag that received an identification number. During processing, all personnel were blind to case-control status of the filters.

Study I A disc, 3.2 mm in diameter, was cut from each blood spot and distributed into deep 96-well plates sealed with AxyMat, Axygen scientific Inc (Union City, CA, USA). Each plate included blank wells that were empty or contained a disc without blood. Blood was eluted from the filter paper by incubation with 100 µl of phosphate buffered saline (PBS). Levels of IgG antibodies directed at infectious agents in the eluates were measured by Enzyme-Linked Immuno Sorbent Assay method (ELISA). Anti CMV and *T. gondii* IgG were

measured by assays from Vitro-Immun Labor Diagnostika GmbH (Oberursel, Germany) and anti HSV-1 and -2 IgG by assays from Focus Diagnostics Inc (Cypress, CA, USA).

Study II Apart from using the aforementioned IgG data, analyses of APP concentration were performed. Another 3.2 mm diameter disc was punched from each blood spot and immersed in 80 μL of PBS containing 1% bovine serum albumin and 0.05% Tween for elution. Eluates were analyzed for the concentration of nine APP using a premixed, magnetic bead-based multiplex panel from Bio-Rad Laboratories Inc (Hercules, CA, USA). Concentrations of the APPs were imputed using Bio-Plex 200 Suspension Array System with Bio-Plex Manager 6.0 software. All personnel were blind to case-control status of the filters during the processing.

4.5 STATISTICAL ANALYSES

Study I The main association investigated was the relation between maternal exposure to the infectious agents and schizophrenia respectively other non-affective psychosis. Exposure status was based on age weighted prevalences for the four pathogens among pregnant women during the years 1975-1985, and was for *T. gondii* 25%, CMV 75%, HSV-1 60%, and for HSV-2 25%. The distributions among control subjects were used to find the cut-off value (the plate normalized optical density value) that corresponded to each of these percentiles allowing maternal IgG levels to be analyzed as dichotomous variables. Antibody levels between 75^{th} and 90^{th} percentiles, and above the 90^{th} percentile for *T. gondii* and between 25^{th} and 50^{th} percentiles, 51^{st} and 75^{th} percentiles, and above the 75^{th} percentile for CMV were also investigated. Odds ratios (OR) and 95% confidence intervals (CI) were calculated by using conditional logistic regression model for matched data. All statistical analyses were made with The SAS software package, Version 9.1 (SAS Institute, Inc., Cary, N.C.).

Study II To explore whether maternal chronic infection with *T. gondii*, CMV, HSV-1, and -2 during pregnancy influence the immune activity in the neonate, median levels of the nine APPs were compared between exposed and unexposed individuals for each of the infectious agents, for controls and cases (non-affective psychosis) separately. As in study I, maternal exposure status was based on the expected age-weighted prevalences among pregnant women in Stockholm during the years 1975-1985: *T. gondii* 25%, CMV 75%, HSV-1 60% and HSV-2 25%. Then the distributions among control subjects were used to find the optical density value (the cut-off value) corresponding to each of these percentiles allowing maternal IgG levels to be analyzed as dichotomous variables. The distribution of APP concentrations was skewed whereby Mann-Whitney U test (M-W) were chosen to calculate p-values of the difference of the median between the two groups (exposed and unexposed). To estimate the effect of maternal exposure and neonatal APP levels on psychosis risk, conditional logistic regression was used to calculate OR and 95% CI. Individuals were classified as either exposed or unexposed to microbial agent (as above) and as having either low APP levels (those in the lowest tertile) or high APP levels (those in the middle and

highest tertiles), again established using the distribution of each APP among controls. The reference was unexposed individuals with high APP levels.

To avoid problems with multiple comparisons and to have a marker of innate immune response we used Principal Components Analysis (PCA) to create an APP score. Information from the nine inter-correlated APPs were collapsed into a single score for each person [117]. Individuals were classified as having either a low APP score (having an APP score below the median) or a high APP score (APP score above the median).

Departure from additivity implicates biological interaction [157]. In other words, biological interaction occurs when the combined effect of two factors is greater than the sum of their individual effect. The relative access risk due to interaction (RERI) can be used to measure departure from additivity using multiplicative models such as logistic regression or cox regression [158]. To identify interaction between low levels of the different APPs and maternal exposure to *T. gondii* respectively CMV in the risk of developing non-affective psychosis or schizophrenia we quantified the relative excess risk due to interaction (RERI). RERI was defined as: $RERI = RR_{11} - RR_{10} - RR_{01} + 1$, assuming that the matched OR can be used alternately of the relative risk (RR). The reference category was defined as those unexposed to both risk factors, i.e., $RR_{00} = 1$. RERI = 0 indicates no interaction. Analyses were made with the IBM SPSS statistics 22.0 (IBM, Armonk, NY, USA).

Study III The main association investigated was the relationship between hospital admission with infection during childhood (0-13 years of age), and non-affective psychosis later in life. Hazard ratios (HR) with 95% CI were estimated using Cox proportional hazards models of time in the study, with non-affective psychosis as the outcome. Time in the study was calculated from the starting date, which was from 14 years of age, until the first hospital admission with non-affective psychosis, date of death, date of emigration, or 31 December 2006, whichever came first.

In addition, to explore the effect of exposure at various ages timing of exposure were divided into four different age periods: first year, 1-3, 5-9, and 10-13 years. To explore if the risk is particular the diagnoses of infection were subdivided into bacterial, viral, and other infection ("other infection" included all non-bacterial and non-viral infections, and unknown or not specified infection), and into CNS and non-CNS infections. Next, we investigated whether the risk of developing non-affective psychosis increased with number of admission with infection during childhood. Additionally, to decide what confounders should be included in the analyses, their associations with the exposure were estimated using logistic regression to calculate OR and 95% CI. Associations between confounders and outcome were calculated by Cox regression (see above). All analyses were made with IBM SPSS statistics 22.0 (IBM, Armonk, NY, USA).

Study IV Children were followed from 14 years of age until first diagnosis of non-affective psychosis, date of death, date of emigration, or 31 December 2011, whichever came

first. Associations between parental hospital admission with infection prior to or during pregnancy and offspring non-affective psychosis were calculated as HR and 95% CI by using the Cox regression model. Pregnancy was defined as first day of last menstrual period (LMP) before pregnancy up until the day of delivery. Trimesters were defined as: 0^{th}: LMP-35days (before uteroplacental circulation is established), 1^{st}: 36-97 days, 2^{nd}: 98-188 days, and 3^{rd}: 189 days-delivery. Associations between maternal infection during pregnancy and childhood infection among offspring were calculated with OR and 95% CI by using logistic regression model. To test for biological interaction between parental infection prior to or during pregnancy and parental psychiatric disorders in the risk of developing non-affective psychosis, the RERI was calculated. Likewise, the RERI was calculated to identify possible interaction between maternal infections prior to or during pregnancy and childhood infections in the development of non-affective psychosis. Statistical analyses were made using IBM SPSS statistics 22.0 (IBM, Armonk, NY, USA).

In all the studies, multiple confounding factors were included in the analyses. These are described in the Methodological consideration section.

4.6 ETHICAL APPROVALS

Ethical approval has been sought and granted from the Regional Ethics Committee of Stockholm, EPN. Paper I, and II: Dnr 03-029 and Paper III, IV: Dnr 2010/1185-31/5. Written informed consent from the participants was required for collection of the neonatal blood samples.

5 RESULTS

5.1 STUDY I

Blood spots from 198 cases and 524 matched controls were collected. Maternal exposure to *T. gondii*, and CMV were associated with schizophrenia (Figure 3), but not with other non-affective psychoses OR 0.8, 95% CI: 0.5, 1.2 respectively OR 1.0, 95% CI: 0.6, 1.5. Adjustments for maternal migration and age ≥35 had no major influence on these estimates. Maternal exposures to herpes virus -1 or -2 were neither associated with schizophrenia (Figure 3) nor with other non-affective psychoses OR 1.0, 95% CI: 0.7, 1.5 respectively OR 1.3, 95% CI 0.9, 2.1.

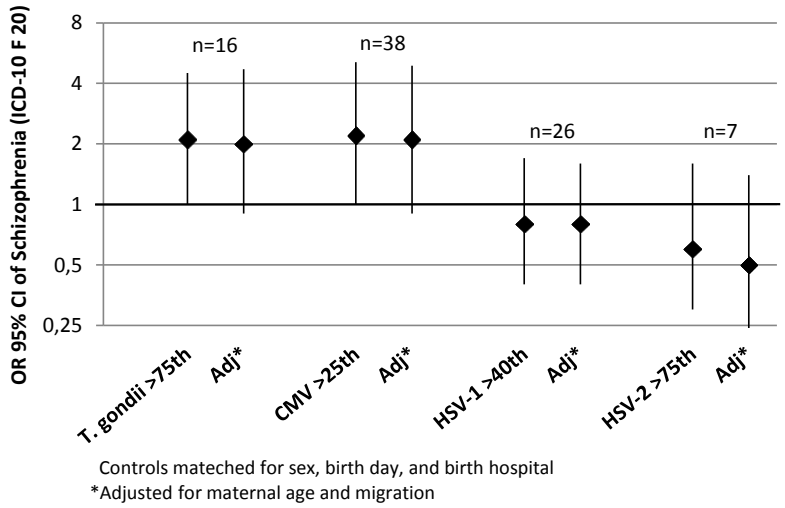

Figure 3. OR and 95% CI of schizophrenia, ICD-10 F20 in relation to levels of IgG directed at *T. gondii*, CMV, HSV-1, and -2. (Study I)

Maternal exposure to *T. gondii* >90th percentile, was associated with a three times higher relative risk of developing schizophrenia than those within the reference range, OR 3.2, 95% CI: 1.0, 9.8. Also CMV antibodies at higher levels (>75th percentile) indicated an increased relative risk OR 2.7, 95% CI: 0.9, 8.2. There was no covariance between *T. gondii* and CMV, indicating an independent association with schizophrenia.

5.2 STUDY II

Acute phase protein levels across maternal exposures among neonates who will not develop psychotic disorder

Children born to mothers with serological evidence of *T. gondii* or CMV exposure had significantly higher levels of each of the nine different APPs in NDBS, and the APP-score were significantly higher as compared to children born to unexposed mothers. There was one exception; the CRP level among CMV exposed mothers did not reach statistical difference in levels as compared to unexposed mothers (Table 4). Maternal exposure to HSV-1 or -2 was not associated with any of the neonatal APP levels (Table S2).

Acute phase protein levels across maternal exposures among neonates who will develop psychotic disorder

Levels of the different APPs or APP scores among individuals who later developed non-affective psychosis or the more restricted diagnosis of schizophrenia did not significantly vary according to maternal exposure to *T. gondii* and CMV (Table 4), or HSV-1 and -2 (Table S2).

Synergistic effect

Among neonates with high APP levels did maternal *T. gondii* infection not affect the odds of developing non-affective psychosis, whereas among neonates with low APP levels there was a pattern of increased odds (Figure 4). When restricting the analyses to cases of schizophrenia only, neonates with low APP levels and maternal reactivity against *T. gondii* had substantially increased odds of developing schizophrenia. Indeed, a low APP-score and maternal exposure to *T. gondii* significantly increased the odds of developing non-affective psychosis OR 2.1, 95% CI: 1.1, 4.0 and schizophrenia, OR 3.6, 95% CI: 1.0, 13.4. Only low neonatal levels of fibrinogen in combination with maternal reactivity towards *T. gondii* reached significant interaction, RERI 1.1, 95% CI: 0.0, 2.2.

Maternal exposure to CMV did not increase the odds of developing non-affective psychosis among neonates with high APP levels (Figure 5). Neonates with low levels of SAP, procalcitonin or tPA and maternal reactivity against CMV had increased odds of developing non-affective psychosis. Restricting the analyses to schizophrenia only, maternal exposure to CMV increased the odds of developing schizophrenia even among those with high levels of APPs, or with a high APP score (Figure 5). Maternal reactivity against CMV and low APP levels (except ferritin) or with a low APP-score was significantly associated with increased odds of schizophrenia development. Once more, being in the lower tertile of fibrinogen together with maternal reactivity towards CMV the OR for schizophrenia increased by 6-fold as compared to those unexposed to CMV, or with high APPs. The odds of developing schizophrenia increased to significantly levels among neonates with maternal CMV exposure and a low APP score, OR 6.0, 95% CI: 1.1, 33.1.

Table 4. Mann-Whitney U-test, p-values of the difference in levels of acute phase proteins among neonates to exposed and unexposed mothers (median, 25th-75th percentile), controls and cases separately. (Study II)

APP	*T. gondii*								
	Controls			Neonates who will develop non-affective psychosis, ICD-10 F20-29			Neonates who will develop schizophrenia, ICD-10 F20		
	Unexposed (n=403)	Exposed (n=122)	p	Unexposed (n=153)	Exposed (n=46)	p	Unexposed (n=30)	Exposed (n=16)	p
a-2-Macroglobulin (ng/ml)	357 (137-615)	594 (352-895)	***	340 (158-589)	420 (129-824)	ns	282 (101-508	368 (128-606)	ns
Haptoglobulin (ng/ml)	6.0 (2.6-21.1)	11.2 (3.3-36.4)	***	6.3 (2.5-15.7)	6.4 (2.3-22.4)	ns	6.2 (2.1-42.1)	6.6 (1.6-23.9)	ns
C-reactive protein (ng/ml)	0.6 (0.2-1.8)	1.1 (0.5-2.4)	**	0.6 (0.2-1.3)	0.8 (0.2-2.2)	ns	0.5 (0.1-1.3)	0.3 (0.1-1.2)	ns
Serum amyloid P (ng/ml)	10.3 (5.1-16.1)	13.8 (7.8-20.5)	***	8.8 (4.7-15.2)	11.0 (6.1-16.0)	ns	8.6 (4.2-13.3)	10.0 (5.1-12.5)	ns
Procalcitonin (pg/ml)	2.1 (0.8-3.3)	3.3 (1.4-5.2)	***	1.9 (0.8-3.2)	2.6 (0.8-4.4)	ns	2.0 (0.3-2.6)	2.2 (0.2-4.4)	ns
Ferritin (pg/ml)	1130 (301-2500)	2350 (665-4760)	***	1430 (331-2830)	1800 (260-4290)	ns	1080 (220-2580)	1250 (150-3920)	ns
tPA (pg/ml)	3.6 (1.3-6.0)	4.8 (2.2-6.9)	*	3.1 (1.0-5.3)	2.2 (0.0-5.6)	ns	2.9 (0.8-4.9)	2.3 (0.0-4.4)	ns
Fibrinogen (ng/ml)	6.8 (2.2-17.9)	14.9 (2.7-46.2)	**	7.6 (2.6-24.9)	3.6 (1.2-20.9)	ns	3.7 (2.7-26.5)	2.6 (0.4-13.0)	ns
Serum amyloid A (ng/ml)	1.6 (0.7-3.5)	2.2 (1.0-5.0)	**	1.5 (0.7-2.9)	1.2 (0.4-2.4)	ns	1.7 (0.7-2.1)	1.2 (0.0-2.3)	ns
APP score	**0.1 (-0.7-0.6)**	**0.6 (-0.8-1.1)**	***	**0.2 (-0.6-0.5)**	**0.1 (-0.6-0.8)**	**ns**	**0.1 (-0.8-0.5)**	**-0.2 (-1.1-0.4)**	**ns**

APP	**CMV**								
	Controls			Neonates who will develop non-affective psychosis, ICD-10 F20-29			Neonates who will develop schizophrenia, ICD-10 F20		
	Unexposed (n=135)	Exposed (n=390)	p	Unexposed (n=48)	Exposed (n=151)	p	Unexposed (n=8)	Exposed (n=38)	p
a-2-Macroglobulin (ng/ml)	348 (83.2-588)	431 (187-698)	**	340 (100-565)	355 (159-678)	ns	343 (128-521)	336 (101-551)	ns
Haptoglobulin (ng/ml)	5.1 (2.3-17.5)	7.2 (2.8-27.8)	*	5.7 (2.2-22.3)	6.5 (2.8-16.1)	ns	1.9 (1.6-37.5)	7.1 (2.4-37.0)	ns
C-reactive protein (ng/ml)	0.7 (0.2-2.0)	0.8 (0.2-1.9)	ns	0.8 (0.2-1.7)	0.6 (0.2-1.4)	ns	0.8 (0.1-0.9)	0.4 (0.1-1.2)	ns
Serum amyloid P (ng/ml)	9.4 (4.3-16.5)	11.5 (6.1-18.0)	*	8.9 (5.8-14.3)	8.9 (4.8-17.0)	ns	7.9 (6.0-10.8)	9.0 (4.2-13.3)	ns
Procalcitonin (pg/ml)	1.9 (4.3-16.5)	2.3 (0.9-3.8)	*	1.8 (0.8-3.2)	2.0 (0.8-3.5)	ns	2.3 (0.4-3.5)	1.9 (0.3-3.5)	ns
Ferritin (pg/ml)	965 (191-2410)	1590 (439-3180)	**	1230 (259-2720)	1730 (335-3130)	ns	960 (250-2080)	1180 (120-2660)	ns
tPA (pg/ml)	3.0 (0.9-5.8)	4.1 (2.0-6.4)	*	2.8 (1.0-4.8)	3.1 (0.5-5.6)	ns	2.4 (0.6-5.3)	2.9 (0.2-4.6)	ns
Fibrinogen (ng/ml)	5.2 (2.0-17.0)	8.2 (2.4-27.3)	*	5.3 (2.5-16.0)	7.4 (2.1-26.2)	ns	3.5 (3.0-20.0)	3.4 (1.4-26.1)	ns
Serum amyloid A (ng/ml)	1.4 (0.6-3.0)	1.8 (0.9-4.1)	*	1.2 (0.5-3.0)	1.6 (0.6-2.8)	ns	0.9 (0.2-2.4)	1.5 (0.6-2.1)	ns
APP score	**0.1 (-1.1-0.6)**	**0.3 (-0.5-0.8)**	*	**0.1 (-0.5-0.6)**	**0.1 (-0.6-0.6)**	**ns**	**-0.3 (-0.6-0.5)**	**0.1 (-1.0-0.4)**	**ns**

*=p<0.05, **=p<0.01, ***=p<0.001, ns=non-significant

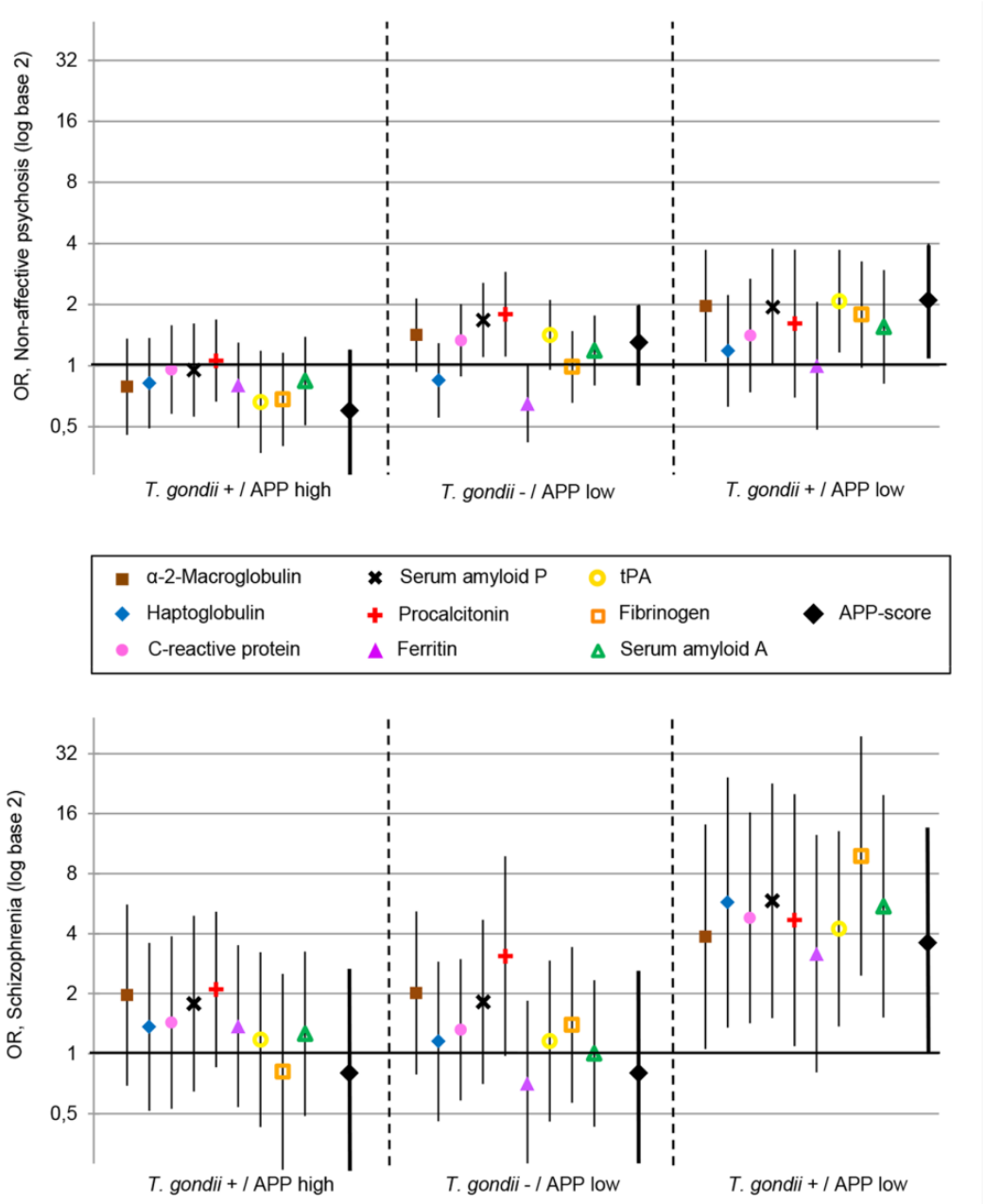

Figure 4. Odds ratios (OR) and 95% confidence intervals of Non-affective psychosis or Schizophrenia according to maternal *T. gondii* exposure status and levels of each APP (lowest 1/3 tertile, and highest 2/3 tertile) and APP-score (below median and above median). (Study II)

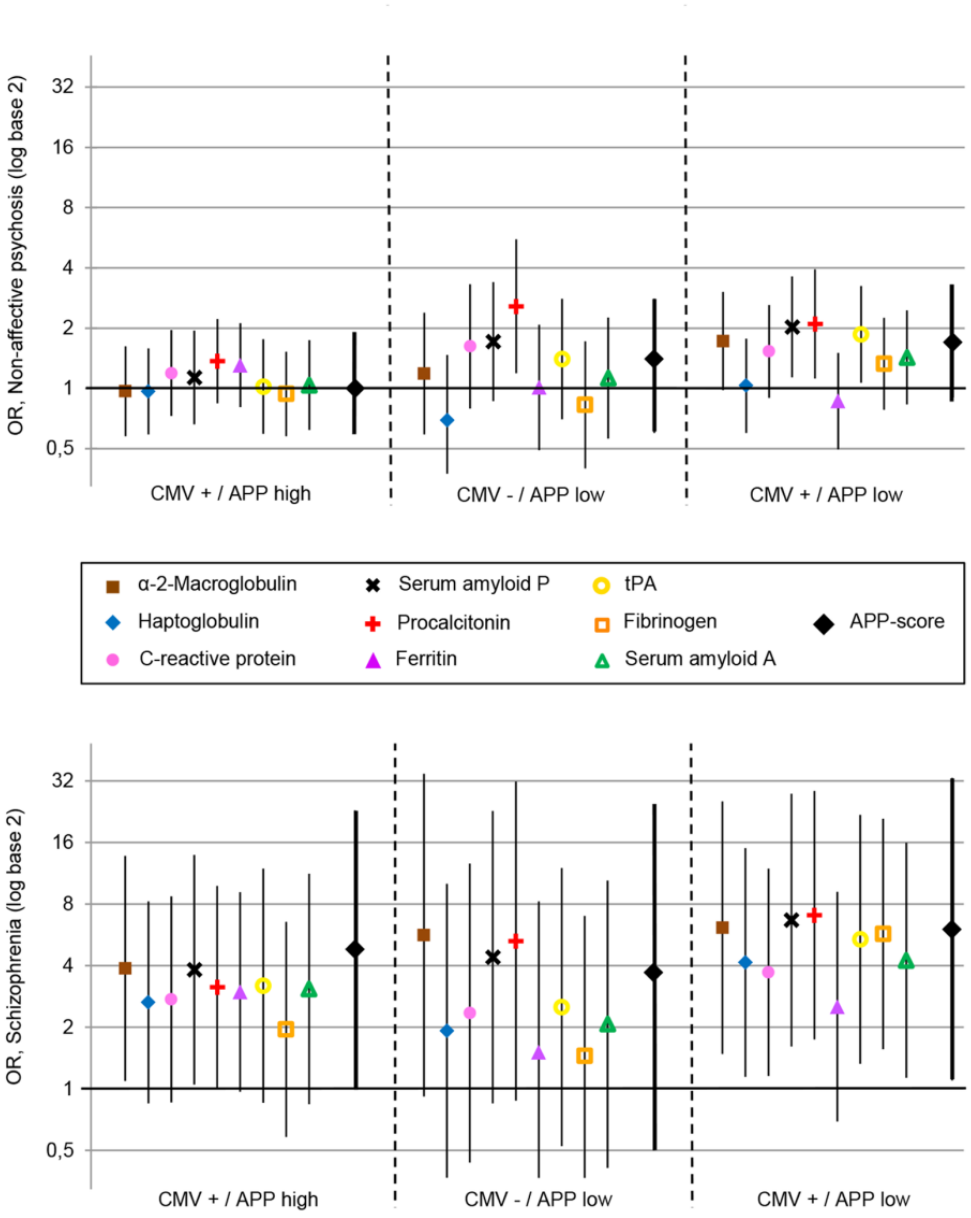

Figure 5. Odds ratios (OR) and 95% confidence intervals of Non-affective psychosis or Schizophrenia according to maternal CMV exposure status and levels of each APP (lowest 1/3 tertile, and highest 2/3 tertile) and APP-score (below median and above median). (Study II)

5.3 STUDY III

Of totally 1 172 879 children followed up in the registers, 4 638 (0.4%) were subsequently diagnosed with non-affective psychoses. There was a modest but significant association between any severe infection during childhood and non-affective psychoses, HR 1.10, 95% CI: 1.03, 1.18 (Table S3). The risk did not differ by age at time of infection.

The association was strongest between hospital admission with bacterial infection and the later development of non-affective psychoses, HR 1.23, 95% CI: 1.08, 1.40, where exposure

to bacterial infection during preadolescence (10-13 years) was associated with the highest risk estimate, HR 1.59, 95% CI: 1.22, 2.08 (Figure 6).

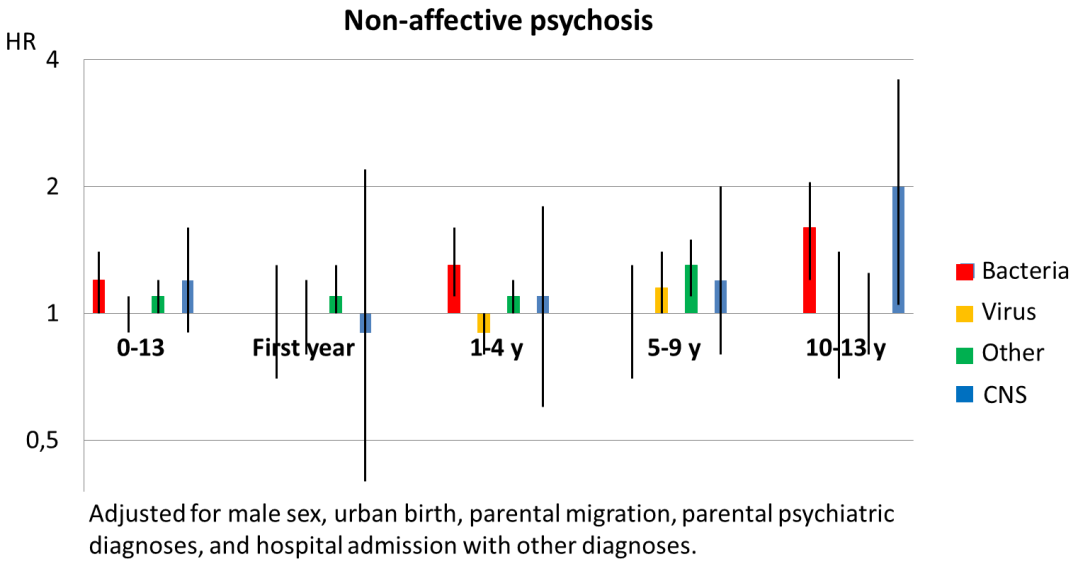

Figure 6. Hazard Ratio (HR) (95% CI) of schizophrenia after hospital admission with infection during childhood 0-13 years. (Study III)

CNS infection was not associated during any age period, with one exception, during preadolescence, HR 1.96, 95% CI: 1.06, 3.65.

Four or more admissions with infection during childhood had slightly stronger risk estimates than one admission, HR 1.37, 95% CI: 1.06, 1.78.

In study III, adjusting for psychiatric disorder in general among parents had stronger effects on the estimate than adjusting only for the more restricted diagnoses of schizophrenia or non-affective psychosis (Table 5).

Table 5. Effect of parental psychiatric diagnoses on the association between childhood infection and non-affective psychosis among offspring. (Study III)

Unadjusted	HR 1.27 (95%CI 1.19-1.36)
Adjusted for:	
parental Schizophrenia (ICD-10 F20)	HR 1.27 (95%CI 1.18-1.35)
parental Non-affective psychosis (ICD-10 F20-29)	HR 1.25 (95%CI 1.17-1.34)
parental any psychiatric diagnose (ICD-10 F00-99)(in the study)	HR 1.18 (95%CI 1.10-1.26)

5.4 STUDY IV

Of totally 1 971 623 children, 8 330 (0,4%) were subsequently diagnosed with non-affective psychoses during follow-up. There was no association between maternal hospitalization for infection during pregnancy and non-affective psychosis among offspring in the fully adjusted model, OR 1.06, 95% CI: 0.88, 1.27. Type of infection (bacteria or virus) or trimester at time of infection did not modify the association (Table S4). In contrast, among individuals whose parents were diagnosed with a psychiatric disorder, exposure to maternal infection during pregnancy was significantly associated with non-affective psychosis, HR 1.30, 95% CI: 1.02, 1.66. Among individuals without parental psychiatric disorder there was no association, HR 0.84, 95% CI: 0.63, 1.11.

Further exploring a familial liability for infection; maternal infection up to 5 years prior to pregnancy was modestly associated with non-affective psychosis in the offspring, (HR 1.14, 95% CI: 1.05, 1.23). Paternal hospital admission with infection prior to pregnancy was not, (HR 0.97, 95% CI: 0.86, 1.09)

There was indication of interaction between maternal psychiatric disorder and infection during pregnancy in the development of non-affective psychosis among offspring, RERI 0.79, 95% CI -0.02, 1.60, especially among mothers who were not hospitalized with infection prior to pregnancy, RERI 1.33. 95% CI: 0.27, 2.39 whereas paternal psychiatric disorder did not affect the associations, RERI 0.14, 95% CI: -0.59, 0.87(Figure 7).

Maternal infection during pregnancy per se or a familial liability for infections (i.e. maternal infection prior to pregnancy) was investigated on the odds of being hospitalized with infection during childhood (Table 6). There was an association both between maternal infection during pregnancy as well as maternal infection prior to pregnancy in the general population. Among individuals who later developed non-affective psychosis maternal infection particularly during pregnancy further increased the odds of childhood admission with infection, OR 2.12, 95% CI: 1.46, 3.07.

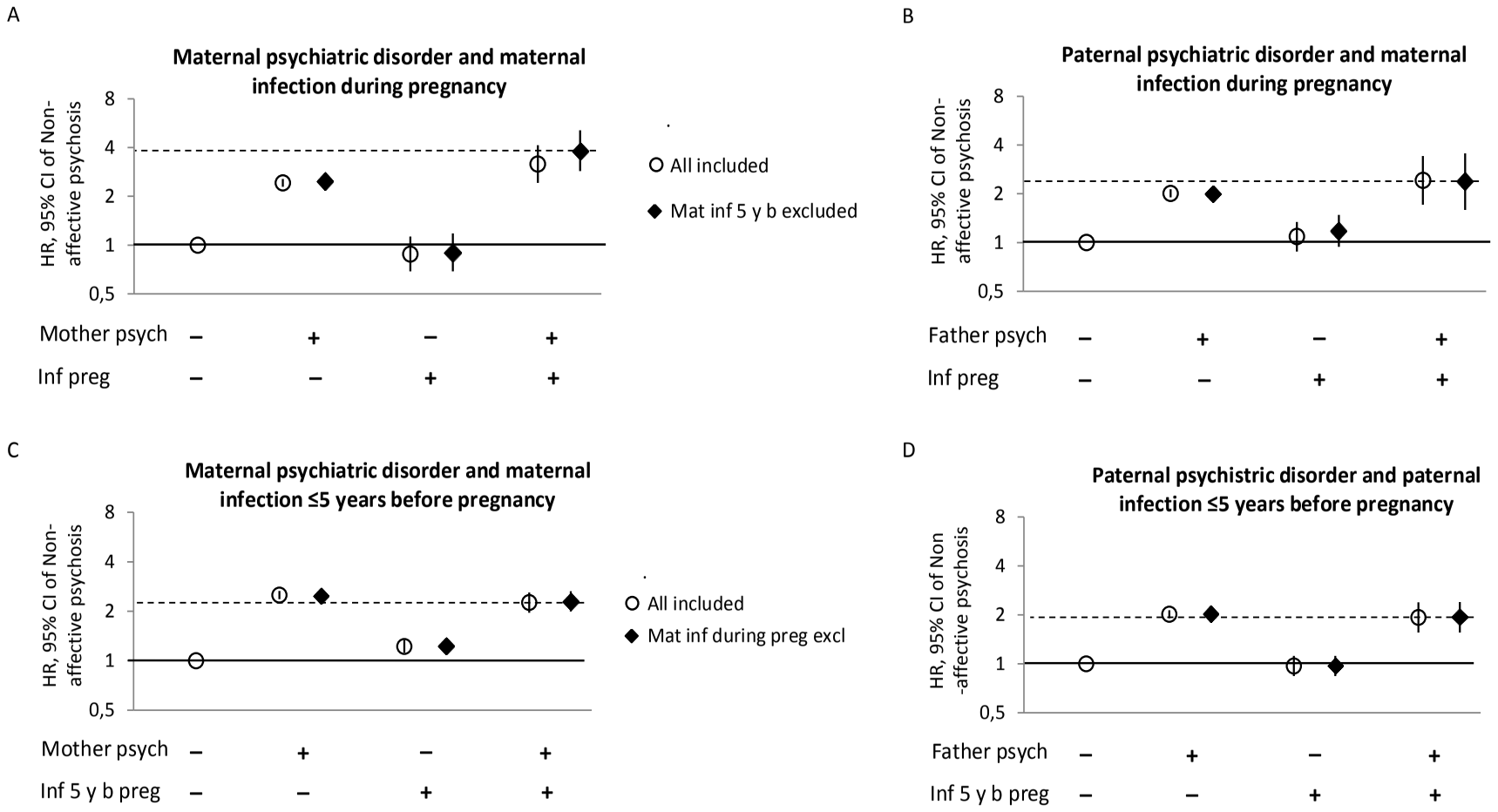

Figure 7. HR and 95% CI of non-affective psychoses among individuals with and without maternal infection during pregnancy, parental infection up to 5 years before pregnancy, and parental psychiatric disorder. Fully adjusted for birth year, sex, urban birth, winter birth, parental age ≥ 35 years, small for gestational age, parent born outside Sweden, low SES, any parent any inpatient care before or during pregnancy except for treatment of infection or psychiatric care. Circles represent the general population and diamonds the restricted model. (Study IV)

Table 6. Associations between maternal infection during pregnancy or maternal infection up to 5 years prior to pregnancy and childhood infection among the total population and among individuals with non-affective psychosis, OR and 95% CI. (Study IV)

	Maternal infection during pregnancy	N, Childhood infection/ No childhood infection	Basic model[1] OR (95% CI)	Adjusted model[2] OR (95% CI)	Maternal infection ≥5 years before pregnancy	N, Childhood infection/ No childhood infection	Basic model[1] OR (95% CI)	Adjusted model[2] OR (95% CI)
Total population	Unexposed	454 825/ 1 493 014	1 (reference)	1 (reference)	Unexposed	422 024/ 1 418 361	1 (reference)	1 (reference)
	Exposed	7 926/ 15 858	1.66 (1.61, 1.70)	1.49 (1.45, 1.53)	Exposed	40 727/ 90 511	1.51 (1.49, 1.53)	1.37 (1.35, 1.38)
Individuals with non-affective psychosis	Unexposed	2 277/ 5 936	1 (reference)	1 (reference)	Unexposed	2 081/ 5 543	1 (reference)	1 (reference)
	Exposed	58/ 59	2.58 (1.79, 3.72)	2.12 (1.46, 3.07)	Exposed	254/ 452	1.49 (1.27, 1.76)	1.29 (1.10, 1.53)

[1]Adjusted for birth year and sex.

[2]Additionally adjusted for urban birth, winter birth, small for gestational age, parental age ≥ 35 years, parental history of psychiatric disorder, parent born outside Sweden, low SES, any parent any inpatient care before or during pregnancy except for treatment of infection or psychiatric care, and for the child during childhood (0-13 y)

There was interaction between maternal infection during pregnancy and childhood infection in the risk of developing non-affective psychosis RERI 0.45, 95% CI 0.03, 0.87, whereas no interaction was found between maternal infection prior to pregnancy and admission with infection during childhood on the risk of developing non-affective psychosis RERI -0.07, 95% CI: -0.25, 0.11 (Figure 8).

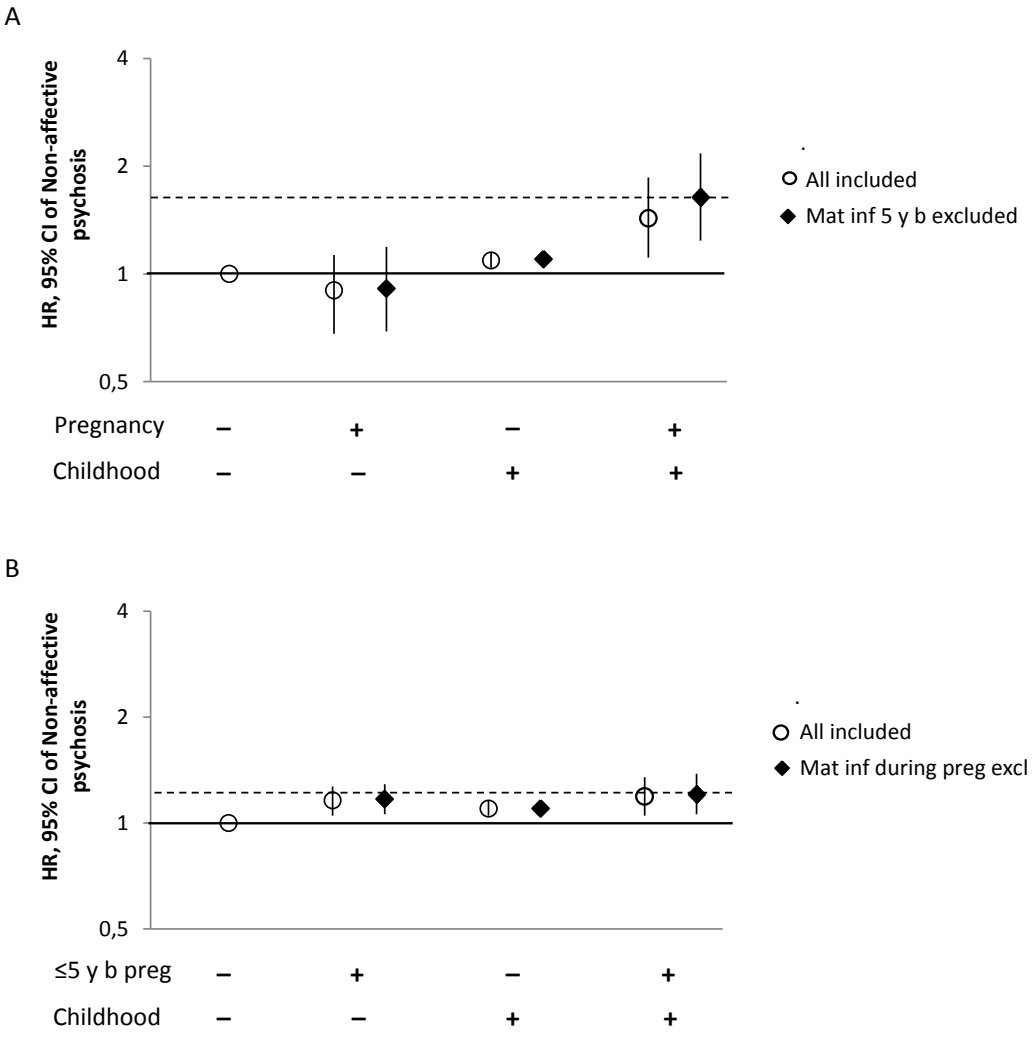

Figure 8. HR and 95% CI of non-affective psychoses according to maternal infection during pregnancy respectively up to 5 years before pregnancy, and childhood infection. Fully adjusted for birth year, sex, urban birth, winter birth, parental age ≥ 35 years, small for gestational age, parental history of psychiatric disorder, parent born outside Sweden, low SES, any parent any inpatient care before or during pregnancy except for treatment of infection or psychiatric care, and for the child during childhood (0-13 y) . Circles represent the general population and diamonds the restricted model. (Study IV)

6 DISCUSSION

6.1 MAIN FINDINGS IN THE CONTEXT OF PREVIOUS STUDIES

6.1.1 Maternal infection during pregnancy

6.1.1.1 Chronic maternal infections

In Study I, we found support for an association between maternal exposure to *T. gondii* and schizophrenia in offspring. This is the third study to find such an association [61,63]. In these studies, including ours, a dose-response relationship was observed, which strengthens the probability of a true association. Increased antibody levels could imply a more recent maternal exposure and perhaps increased pathogen or immune component levels during the pregnancy, which could have greater impact on the fetus. Although we aimed to have a larger sample size than previous studies, we had a large drop-out and ended up with a study population similar to the previous ones. Thus, all studies, including the two studies that did not find support for an association [56,58] have had low power to detect a true difference between exposed and unexposed mothers.

Apart from MIA and a potential transmission of the pathogen, the observed associations between elevated maternal IgG antibodies and psychotic disorder could be interpreted as markers of a high load of the pathogen in the environment/family. For example, high levels of maternal *T. gondii* IgG could be a marker of cat ownership [159], which may imply an increased probability of later exposure, with a more causal association. Indeed, individuals with schizophrenia have increased levels of antibodies directed at *T. gondii* [160]. This theory could also be true also for infections in general, e.g., bad hygiene habits in the family may imply increased susceptibility of infections overall.

This is the first study to find an association between chronic maternal CMV infection and schizophrenia in offspring. Unfortunately, the previous two studies that did not find support for an association had low power to detect a difference, as was also the case in our study, [56,62]. In addition, as the worldwide prevalence is high but varies considerably between decades, countries and in different age groups, it is important to consider the prevalence in the study population when determining the cut-off level for negative and positive exposure status. It is possible that the two negative studies misclassified the exposure (comparing exposed individuals with a mix of unexposed and exposed individuals), thus underestimated the association.

The biological mechanisms behind the schizophrenia risk associated with chronic maternal CMV infection during pregnancy are unknown. We have for example no knowledge whether the pathogen is transmitted to the fetus. Recently, an increased prevalence of CMV DNA was found in neonatal blood from children diagnosed with autism. Interestingly, these children had no signs of congenital CMV infection [161]. There are reports of a link between genetic variability in the MHC-region as well as in the TNF-region, CMV infection and schizophrenia risk [148,162,163], which suggest the involvement of immunological mechanisms.

Several studies indicate an altered immune response among patients with schizophrenia [118,132,164]. Such deficiencies seem to be already present at birth. Gardner *et al.* recently reported that neonates who will develop non-affective psychosis have low levels of some APPs [117].

In Study II, we investigated whether maternal infection during pregnancy affected neonatal levels of APP. We found that those in the control group who had mothers with chronic *T.gondii* and CMV infection had elevated APP levels. In contrast, neonates who later developed non-affective psychosis did not have elevated APP levels. Interestingly, cases and controls with unexposed mothers had the same low levels of APP. Thus, the low levels among cases indicate a deficient response to maternal infections rather than low circulating levels. In addition, we found evidence of *T.gondii* increasing the risk of non-affective psychosis only among neonates who did not have elevated APP levels. Hence, individuals who later develop psychotic disorder seem to respond inadequately to some chronic maternal infections which increase the vulnerability to these exposures. These findings support the hypothesis of a deficient innate immune response in the aetiology of schizophrenia [131]. However, these findings should be interpreted with caution, though our studies had several methodological weaknesses, which are described in the Methodological considerations section.

Including Study I, three studies have found and three have not found support for an association with HSV-2. Apart from methodological limitations in all studies, these discrepancies could be explained by variation in circulating HSV-2 genotypes on different continents and during various decades [165].

We found no evidence of an association between chronic maternal HSV-1 infection and schizophrenia which is in accordance with all previous studies [56,62,63]. Although the reliability of these studies is limited, it nevertheless appears that chronic maternal HSV-1 infection during pregnancy is not a risk factor for non-affective psychosis.

6.1.1.2 Maternal hospital admission with infection

In Study IV, we did not find support for an association between maternal infection during pregnancy and non-affective psychosis in offspring overall. Nor did we find evidence of infections restricted to only bacteria respectively only virus to increase the risk of psychosis. The two previous studies that reported positive associations with respiratory respectively bacterial infections had few exposed cases [99,102]. The study investigating *any* infections during pregnancy in a large population based birth cohort found a modest association, RR=1.2 [103]. Notably, neither of these studies were adjusted for important confounders such as parental migration, SES, psychiatric history in the family and care-seeking habits which could explain the differing results. In fact a psychiatric (not only psychotic) history and care-seeking habits in the family (parental admission to hospital before and during pregnancy) had the largest attenuating effects on our estimates. Our crude estimates were similar to those in the latter study.

To distinguish the specific effect of infection in utero from the genetic overlap of liability for both infection and schizophrenia, we compared the risks associated with maternal and paternal infection *before* and *during* pregnancy. Only one previous study has investigated the association between both maternal and paternal infection and offspring schizophrenia [103].

We found a modest familial susceptibility to severe infection; maternal infection prior to pregnancy was weakly associated with offspring psychosis. However, the fact that no evidence of an association was found for paternal infection before pregnancy speaks against a general familial susceptibility to infections among patients with non-affective psychoses, as was reported for patients with schizophrenia by Nielsen *et al.* [103]. That study, however, included parental infections *after* the birth of the index child where infection transmission within the family may have occurred [104,105], whereby an effect specific to the foetal period could not be ruled out.

We found a synergistic effect between maternal psychiatric disorder and maternal infection *during* pregnancy, but not *before* pregnancy in the risk of non-affective psychosis in offspring. These findings imply a specific intrauterine effect of maternal infection per se among vulnerable mothers. The evidence of interaction is discussed further in the Two-hits section below.

6.1.2 Childhood infection

In Study III, we found a moderate association between hospital admission with *any* infection during childhood (0-13 years of age) and non-affective psychosis later in life. Bacterial infection and infection in the CNS, especially during the pre-adolescence period, had the strongest association after adjusting for infections during previous age periods. The same infection diagnoses were used in a Danish population based study, published concurrently with our study [166]. The Danish study found slightly stronger associations between any infections at 10-14 years of age and schizophrenia. However, they had not adjusted for care-seeking habits which had an attenuating effect on our estimates. Interestingly, similar to our results, the strongest associations were with bacterial infections.

The mechanisms behind these findings are not known. However, the associations observed with bacterial infection could mirror the effects of treatment with antibiotics. As mentioned previously, a dysfunctional microbiom in the gut may be involved in the aetiology of neuropsychiatric disorders [144]. Regarding the associations specifically during the pre-adolescence period sparse support can be found in the literature: During the pre-adolescence period, the normal pruning processes of the brain imply reduction in plasticity and decrease in synaptic contacts. This process is suggested to be exaggerated and lead to the prodromal symptoms seen in schizophrenia patients [45,167]. Additionally, it is recognized that some infections are more prevalent during adolescence, indicating that this period is sensitive for infection [168]. Thus, studies assessing infection during these years in relation to prodromal symptoms are of importance.

6.1.3 Multiple hits?

In Study IV, we found support for the two-hit theory of schizophrenia, as in our data infection during foetal life was only associated with psychosis development in conjunction with genetic vulnerability. Genetic vulnerability *per se* was strongly associated with non-affective psychosis development, but the association became even stronger in combination with infection during foetal life. Similarly, Clarke *et al.* reported on synergism between parental history of psychotic disorder and maternal pyelonephritis during pregnancy in the risk of schizophrenia in offspring [101]. Parental psychiatric disorder is a crude marker of genetic vulnerability. Although there is genetic overlap between many neuropsychiatric disorders, and they share many environmental risk factors [169], relatively few patients with schizophrenia have parents with psychiatric disorder [6]. Moreover, mothers with psychiatric disorder may have a different intrauterine environment in terms of medication, BMI, or life-style, which could explain the observed interaction. We adjusted for care-seeking habits, SES and parental migration, which should take care of some of the confounding effect, but there is a risk of residual confounding. Interestingly, specifically maternal but not paternal genetic load was part of the association.

Consistent with a "two-hit" model is that prenatal environmental factors or stressors could have priming effects or sensitize the brain or the immune system, increasing the vulnerability and the susceptibility to later insults [119,170]. In Study IV, we found that foetal and childhood infection acted synergistically in the development of non-affective psychosis. This suggests that among individuals who will develop non-affective psychosis later in life, prenatal infection affects the child for a greater insult by a second stressor in terms of childhood infection. An interesting question is whether a carrier of genes susceptible to psychotic disorder (e.g., in the MHC-region) who is exposed to severe infections on *multiple* occasions is at higher risk of developing the disease than a carrier who has been protected against serious infections, suggestive of not only a "two-hit" but a "multiple-hit" model. Unfortunately, we had too few cases in the strata to investigate genetic vulnerability in terms of parental psychiatric disorder in conjunction with *both* foetal and childhood infection.

6.1.4 The pathophysiology of psychosis in context of infection during foetal life and childhood

The pathoaetiology of schizophrenia is unknown but the hyperdopamine and hypoglutamate hypotheses are the most recognized [171,172]. In the 1950s Carlsson *et al*., discovered DA in the brain, which led to the dopamine hypothesis in schizophrenia [171,172]. This hypothesis gained clinical support due to observations of drugs that stimulate the DA-system (e.g., amphetamine), inducing psychotic symptoms in healthy individuals, as well as observations that antipsychotic drugs that reduce the positive symptoms present in schizophrenia block the DA D2-receptors [173,174]. However, the modest reduction in negative symptoms induced by antipsychotic drugs eventually led to the dopamine-imbalance theory of schizophrenia involving impaired DA D1-receptor signaling [175,176]. Later, Phencyclidine (PSP) and the anaesthetic drug Ketamine were observed to cause both positive and negative symptoms as

well as cognitive impairments similar to those seen in schizophrenia [177]. Subsequently, the NMDA receptor hypofunction hypothesis was proven when PCP and Ketamine were found to inhibit the glutamate NMDA-receptor [178]. More recently, it has become increasingly evident that the altered neurotransmission in schizophrenia involves an intricate balance between inhibition, and activation as well as interactions between not only dopamine and glutamate but also other neurotransmitters such as GABA, and acetylcholine and their receptors in complex neural circuits, reviewed in [179].

6.1.4.1 Kynurenic acid

Kynurenic acid (KYNA) is potentially an underlying pathophysiological mechanism of schizophrenia development that has been extensively investigated [180]. The amino acid Tryptophan, an essential brick in protein synthesis, is degraded through the kynurenine pathway and KYNA is one of the end products. KYNA has similar characteristics to PCP and Ketamine in that it blocks the NMDA-receptor [181]. Of relevance for schizophrenia development is the fact that experimental studies have revealed that elevated KYNA increases DA-activity [182]. Moreover, elevated levels of KYNA have been found in the cerebrospinal fluid (CSF) and prefrontal cortex in small samples of schizophrenia patients [183-185]. Interestingly, many infections are associated with the degradation of Tryptophan through the kynurenine pathway, and elevated levels of KYNA have been measured in cerebrospinal fluid (CSF) in conjunction with infection [186,187]. Speculatively, KYNA could thus be a link between infection and psychotic disorder.

6.1.4.2 Vagus nerve

The brain is regulated from the periphery by means of both humoral and neural pathways. Cytokines are involved in the humoral dialogue between the peripheral immune system and the CNS, where the vagus nerve is the neural actor in the cross-talk between the two [188]. The vagus nerve is part of a neural circuit called the cholinergic anti-inflammatory pathway (CAP) in which nerve signals from the periphery to the brain and back again regulate inflammation at the periphery [189]. Cytokine receptors on the efferent arc of the vagus nerve become activated on infection/inflammation and transfer signals along the axon of the nerve via the brain stem to central parts of the brain [188]. What happens when the signal reaches the central parts of the brain is not completely understood, but neuroimaging studies report change in blood flow and activity in many of the brain regions implicated in neuropsychiatric disorders [190]. It can be speculated whether the vagus nerve is a link between infection and brain alterations.

6.1.5 Additional findings

One finding that may be of interest outside the research area of this thesis is that chronic maternal *T. gondii* and CMV infection seem to affect the innate immune response in the neonate. So far only chronic maternal parasite and helminthe infections common in developing countries have been reported to affect the child's immune response [119]. These alterations are independent of pathogen transmission and are present for several years.

6.2 METHODOLOGICAL CONSIDERATIONS

6.2.1 Study design

6.2.1.1 Case-control studies

In a case-control study affected individuals (cases) are selected from a source population, after which a number of unaffected individuals (controls) are randomly selected from the same population to serve as a comparison group. A case-control study is effective when there are few resources, such as biological samples available (Study I and II). The disadvantage is that case-control studies are very sensitive to selection bias and may be inefficient for rare exposures. It is not possible to estimate risks or rates from a case-control study as only a sample is selected from the population. Instead, an odds ratio (OR) can be estimated, defined as the odds of being a case if exposed, compared to the odds of being a case if not exposed. However, the OR can be interpreted as a relative risk if the outcome condition is rare [157].

6.2.1.2 Cohort studies

In a cohort study a group of people who share similar characteristics, e.g., born during a particular time period and in a specific geographic area, are followed up until a fixed end point, e.g., death or a given date. A cohort study can either be prospective or retrospective. If prospective, data about the study population are collected before the start of follow-up, which minimizes measurement errors and increases the possibility to select as many potential confounders as possible. A prospective cohort study is not suitable for investigating rare conditions or those that takes time to develop or are rare though it tends to become very expensive and time consuming. In a retrospective cohort study, data are collected after having been recorded in, for example, a register, making it time efficient and more convenient. The disadvantage is that the study is restricted to pre-existing data of exposure, outcome and confounding factors. However, the Swedish registers used in this thesis (Study III and IV) include just about the total population and collectively information on many factors.

6.2.2 Random errors

The precision of a study reflects the certainty of an estimate not being affected by random errors. Random errors are due to chance and related to the variability in the data. The larger the sample size the less is the likelihood of random errors. The CI reflects the degree of certainty (e.g., 95%) that the point estimate will lie within the interval if the study was replicated repeatedly. The same formula is used to estimate both the p-value and the CI. Hence, a p-value <0.05 is analogous to a 95% CI that does not including the null value. Study I and II are fairly small studies and the likelihood of random errors is therefore large, with wide confidence intervals.

6.2.3 Systematic errors

6.2.3.1 Selection bias

Selection bias is caused by how the participants are recruited to the study and occurs when the association between exposure and outcome differ between those included in the study and those who are not (the remaining source population). The risk of selection bias is particularly high in case-control studies.

In study I and II, cases were retrieved from both in- and outpatient registers. However, those treated in primary care only are not included. There is therefore a limited risk of selection of more serious cases.

In study I and II an inclusion criterion for the controls was no in-patient treatment for a psychiatric diagnosis, which made them "healthier" than the source population and may have introduced selection bias. The controls were no longer representative of the source population from which the cases were selected. As a psychiatric disorder may be associated with the exposure, the exposure tendency among the controls might have been lower than in the source population and the association overestimated. However, it is possible that the controls had been treated in primary care or psychiatric outpatient clinics. About 25% of individuals with non-affective psychosis are only found in out-patient care registers in Stockholm County Council [28]. Moreover, less acute psychiatric disorders such as depression are mainly treated in outpatient care [1].

A large drop-out among both cases and controls occurred in study I and II and could have led to selection bias had it occurred in a systematic manner. However, there was no selection of less serious cases, as the proportion of schizophrenia of those who agreed and those who declined to participate was the same. In the final sample the proportion of immigrants was the same in the control group and the source population. In addition, cases and controls who declined to participate had the same distribution of birth year and sex as the cases and controls included in the study, thus there was no indication of selection bias.

The risk of selection bias in total population cohort studies (Study III and IV) is limited.

6.2.3.2 Information bias

Information bias occurs due to misclassification or measurement errors. When the misclassification is equal among the two study groups (cases and controls, or exposed and unexposed) it is termed non-differential (random) misclassification. This type of misclassification is common in observational studies and weakens the association. If the misclassification differs between the two groups it may bias the estimate in both directions. In this thesis the main sources of misclassification are the ascertainment of exposure to infection in the biological samples, the criterion of only hospital treated infections and the validity of the diagnoses in the registers.

Misclassification of the exposure

In study I and II, there is a risk of exposure misclassification as there are no predefined antibody levels corresponding to positive exposure. Instead, age-weighted seroprevalences of the different pathogens among pregnant women during the period 1975-85 were used to find the positive and negative exposure status limit in terms of antibody levels. We have no reason to believe that these misclassifications differed between cases and controls, whereby the results should not have been affected to any great extent.

Laboratory analyses always involve a risk of faulty instruments or human error. In study I and II all laboratory analyses were blinded to case control status. Thus, there is no reason to believe that these potential errors are differential.

In study III and IV diagnoses of infection were collected from the NPR. Validation studies that compared the diagnoses recorded in the NPR and medical records have shown a positive predictive value of about 80% for somatic diagnoses [191].

In study III and IV we only had information on infections that required hospital treatment. A number of factors associated with psychotic disorder, such as social inabilities, and a small social network, may increase the probability of being hospitalized with infection given the same degree of severity of infection as compared to families without such problems. This would imply a higher probability of cases being detected in hospital registers. Circumstances such as social inability could also imply *less* care-seeking tendencies in the family. Consequently, there were risks of differential misclassification, which could have affected the estimates in either direction, in study III and IV.

Misclassification of the outcome

The diagnoses of non-affective psychosis were based on register data rather than individual assessment. In study I and II the diagnoses of non-affective psychoses were collected from both the NPR and the PVS. The PVS has been validated by comparing registered diagnoses of non-affective psychoses with medical records [20].

In study III and IV, diagnoses of non-affective psychosis were collected from the NPR. Validation studies that compared the diagnoses recorded in the NPR and medical records have shown a positive predictive value of 85-95% for psychiatric diagnoses [191-193]. In study III, only cases with inpatient care were included. From 1995, the Swedish Mental Health Care reform led to a shift from institutionalized psychiatric care to treatment in outpatient care. Thus, there is a risk of underestimation of the cases in study III. In study IV, both inpatient and outpatient care were included. However, the outpatient register did not have complete coverage until 2005, whereby there is a risk that all incident cases were not captured [28]. The underestimation of the cases in study III and IV probably led to dilution of the estimates.

6.2.3.3 Confounding

Confounding is a common systematic error in observational studies and threatens the validity by interfering with the effect of the exposure on the outcome. By definition a confounder has to be associated with both the exposure and the outcome, but cannot be present on the causal pathway between them (i.e., cannot be an effect of the exposure). There are several ways of dealing with confounding in the study design, e.g., randomization, restriction, and matching as well as in the analysis, e.g., matching, stratification, and regression modelling. All studies in this thesis used regression modeling to adjust for multiple confounding variables.

Confounding factors considered were based on à priori knowledge of potential risk factors to the outcome and associated with the exposure.

Study I and II The controls were matched to the cases in terms of sex (infection and non-affective psychosis more common among males), birth date (time dependent birth excess in relation to exposure status and outcome), and birth hospital (differing hospital routines may affect both exposure and outcome) to minimize confounding by these factors. In addition, maternal age ≥35 years was considered a confounder, as older mothers tend to have higher levels of IgG directed at *T. gondii*, CMV, HSV-1 and -2 [84,93,194,195]. Having a mother who was born outside Sweden was also considered a confounding factor. Being born outside Sweden could imply a different exposure environment and thereby IgG levels that differ from those of Swedish mothers [194,196]. The effects of gestational age and mode of delivery were also evaluated. The IgG levels in a newborn baby increase with gestational age [197]. During vaginal delivery a boost of IgG is transferred from the mother to baby, which does not occur during a caesarean section [198]. However, caeserean section was associated with neither the exposure, nor the outcome, and gestational age did not differ between cases and controls. Though we were already underpowered, these variables were not included in the analyses. Unfortunately, we did not have information on parental psychiatric disorders.

It was not possible to adjust for factors that may confound the association between maternal exposure to infection and neonatal levels of acute phase proteins, such as medication or disease, in study II.

Study III To decrease the bias due to confounding, while not losing precision of the estimates by including numerous confounders, we decided that the confounding factor had to have an association with the exposure and outcome of at least OR/HR 1.2 as well as a prevalence of at least 5% in the study population (Table 7). Finally, the confounders included in the adjusted model were male sex [199], born in municipality with ≥200 000 inhabitants [200], mother or father with psychiatric disorder (ICD-10 F00-99) [201], mother or father born outside Sweden [196], and hospital admission during childhood (except with infection or with psychiatric disorder).

Table 7. Prevalences of potential confounders and their association with non- affective psychoses, and childhood infection, HR and OR, 95% CI. (Study III)

		Non-affective psychosis HR (95% CI)	Childhood infection OR (95% CI)	Prevalence (%)
Male sex		1.4 (1.3-1.4)	1.3 (1.3-1.3)	51
Born Dec-May		1.1 (1.0-1.1)	1.0 (0.9-1.0)	52
Urban birth		1.4 (1.3-1.5)	1.2 (1.2-1.3)	14
Parental migration		2.0 (1.8-2.1)	1.2 (1.2-1.2)	9
Parental age ≥35	Maternal	1.4 (1.3-1.5)	1.0 (1.0-1.0)	8
	Paternal	1.4 (1.3-1.5)	1.0 (1.0-1.0)	18
Parental psychiatric disorder	Maternal	3.1 (2.9-3.4)	1.5 (1.5-1-5)	8
	Paternal	2.6 (2.4-2.8)	1.3 (1.2-1.3)	9
Socio economic status (SES)	Parent unemployed	2.4 (2.2-2.7)	1.3 (1.3-1.4)	3
	Household receiving social welfare benefits	1.5 (1.4-1.6)	1.1 (1.1-1.1)	35
Inpatient care with other diagnoses[1]		1.3 (1.2-1.4)	2.1 (2.1-2.1)	34
Maternal infection during pregnancy		1.6 (1.2-2.0)	1.8 (1.7-1.9)	0.9

[1]Hospital admission with all diagnoses except a diagnosis of infection or a psychiatric diagnosis

Study IV In the basic model all risk ratios were adjusted for sex of child, birth year standardized into centered birth year (departure from mean year=0 (referent)), and c-birth year squared to adjust for period and cohort effects. In this study the strategy was to include as many potential confounding variables as possible as we had enough power: child born December-May [11], in municipality with ≥200,000 inhabitants in 1980 [200], small for gestational age (SGA) [202], either parent ≥ 35 years at time of birth [195], either parent born outside Sweden [196], low socioeconomic status (SES) in terms of single-parent household, disposable family income on an individual level, and highest educational level of parents [203]. In addition either parent with a diagnosis of psychiatric disorder (ICD-10 F00-99) [201], and familial tendency to seek hospital care, or to be hospitalized were considered in terms of hospitalization of either parent with any diagnosis other than infection or of psychiatric origin up to 5 years before, or during pregnancy. Associations between the various covariates and exposures (maternal infection up to 5 years prior to pregnancy, maternal infection during pregnancy, and infection during childhood), and the outcome of non-affective psychosis can be found in Table S5.

6.2.3.4 Right censoring

Right censoring takes place when, at the end of follow-up, the event (outcome), for example a diagnosis of non-affective psychosis, has not yet occured. In all the studies in the thesis the study participants were between 28 and 33 years old at end of follow-up. Consequently, the event might have eventually occurred had the follow-up time been longer.

6.2.4 Generalizability

Generalizability, also termed external validity, is the extent to which the results can be applied in a different setting such as a population from another country. Studies using a total population usually have good generalizability. However, in all studies the cases were diagnosed with non-affective psychosis before the age of 28-33 years of age. Consequently, the results probably cannot be generalized to non-affective psychosis developed at an older age.

In addition, the study populations were born between 1975 and 1997 whereby their mothers were exposed to the specific strains of infections existing in Sweden at that time. Our results can be applicable to other Nordic countries but must be cautiously interpreted in relation to other regions and time periods.

Moreover, in terms of clinical diagnosis of infection we only had data on in-patient care and consequently only severe infections. Thus, our results cannot be generalized to less severe infections.

7 CONCLUSION AND FUTURE DIRECTIONS

Although infection early in life is an acknowledged risk factor for schizophrenia, no consistent support can be found in the literature. It is a challenging task to explore early risk factors for psychotic disorder, as it generally takes two to three decades for the disease to develop and psychotic disorder is a relatively rare condition. In addition, many factors, including genes and life-style related to both infection and psychosis must be included in the analysis in order to isolate the role of infection. In this thesis, a combination of Swedish total population register linkages, which allow examination of total population samples with prospectively gathered data on a multitude of individual parameters and with relatively long follow up, and biological samples from the neonatal period from a subset of individuals, allowing investigation of more specific research questions were used.

We found no support for an association between maternal admission for infection during pregnancy and non-affective psychosis in the offspring, while hospital admission due to infection during childhood was modestly associated with subsequent non-affective psychosis. Similar results have been observed in a Danish setting [103,166]. Associations with various infections and immunological alterations have guided the hypothesis towards host factors common to all infections rather than the particular infection *per se* in the causal pathway. Assuming that the theories of a host response to infection, such as maternal immune activation or a deficient immune response in the child, are correct, why do we and others find such modest associations with any kind of severe infection? Considering the fact that we investigated infections requiring hospital treatment, which are presumably more severe and should serve as a solid marker of individual immunologic host factors, one could expect the associations to be stronger.

However, maternal infection during pregnancy, but not prior to pregnancy was associated with increased risk among the offspring of a susceptible group of mothers with psychiatric disorder. The association remained significant after adjusting for multiple socio-environmental factors, indicating that the direct intra-uterine effect of exposure to infection in combination with genetic susceptibility is of relevance.

Maternal infection with the parasite *T. gondii* during pregnancy doubled the relative risk of offspring schizophrenia. Two previous studies have reported similar results, whereby these findings can be considered relatively robust [61,63]. A novel finding was the association between maternal exposure to CMV and schizophrenia. More recently, GWAS have observed interactions between gene variants and maternal CMV infection in psychosis development [147,149]. We found no evidence of associations between HSV-1 and -2. Thus, specific infections or related factors may be more hazardous than others in terms of the risk of psychotic disorder. Another novel finding was that exposure to maternal *T. gondii* seemed to be harmful only in neonates who did not develop elevated APP levels. Similar observations were made of maternal CMV infection. These findings suggest that the neonatal immune response is crucial in relation to the risks associated with infections during foetal life. Our result requires

replication, in larger studies with a longer duration of follow-up and preferably from other populations. In addition, ascertainment of transmission of the pathogen would be valuable.

Regarding childhood infections, bacterial and CNS infections during the pre-adolescence period indicated the strongest associations with non-affective psychosis. A novel finding was that the risk associated with childhood infections was stronger in conjunction with infection during foetal life, indicative of a "second-hit" model. In the future, longitudinal studies exploring a "multiple-hit" model, i.e., the risks associated with *successive* exposures to infection during foetal life, childhood and adolescence, and their interaction with genetic variations, should have large explanatory power. Furthermore, it would be valuable to investigate a wider spectrum of infections ranging from the common cold to more serious infections that require hospitalization as well as antimicrobial treatment on the risk of psychosis development.

In conclusion, the results of this thesis do not support any strong associations between infection early in life and non-affective psychosis overall. However, infection during foetal life seems to be a relevant risk factor in conjunction with genetic vulnerability and immune processes as well as with later exposure to infection. Notably, we had limitations in our studies whereby our findings must be interpreted with caution.

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10 SUPPLEMENTARY MATERIAL

Table S1. All diagnoses of infection in ICD-8, -9, and -10[1].

Bacterial infection in CNS[2]	ICD-8	013.00-013.99, 027.01, 036.00, 090.40, 094.00-094.98, 320.00-320.80, 322.00-322.03, 392.99
	ICD-9	013-X, 036A, B, 090E, 094-X, 320-X, 324-X, 392-X
	ICD-10	A02.2 (if G01), A17-17.9, A22.8, A32.1, A39.0, A39.8 (if G05), A50.4 (if G05.0 or G01), A51.4 (if G01), A52.1 (if G05.0, G01 or F02.8), A54.8 (if G07 or G01), A69.2 (if G01), G00-00.9, G01, G04.2, G05.0, G06-06.2*, G07, I02-02.9
Viral infection in CNS	ICD-8	040.00-043.99, 045.00-046.99, 052.00, 054.04, 062.00-065.99, 071.99, 072.01, 075.02, 079.20, 474.99
	ICD-9	045-049X, 054D, 052B, 053A, 053B, 055A, 056A, 072B, C, 321B-H, 323A, 323C, D
	ICD-10	A80-89, B00.3-00.4, B01.0-01.1, B02.0-02.1, B05.0-05.1, B06.0, B26.1-26.2, G02.0, G05.1
Other/unknown infection in CNS	ICD-8	084.00, 320.88-320.99
	ICD-9	006F, 321A, 321W
	ICD-10	A06.6, B37.5, B38.4, B43.1, B45.1, B46.1, B50.0, B57.4, B58.2, B60.2, B69.0, B83.2, G02.1-02.8, G04, G04.9, G05.2
Bacterial non-CNS infection	ICD-8	000.01-005.99, 008.00-008.39, 010.99-012.97, 014.00-018.98, 020.00-027.00, 027.08-027.98, 030.00-035.99, 036.10-039.98, 073.99, 076.99, 079.30, 080.99-083.99, 088.99-089.90, 090.00-090.30, 090.50-093.99, 095.00-097.99, 098.00-099.91, 100.00-104.98, 361.00-361.09, 362.02, 366.00, 369.00, 380.00-380.01, 382.00-382.99, 383.00-383.99, 390.97-391.99, 421.00, 461.00-461.09, 462.02, 463.01, 481,99-482.98, 501.99, 508.00-508.02, 510.01-510.09, 511.10, 513.99, 522.50, 527.30, 528.00, 528.30, 540.03, 562.00-562.19, 566.00-566.01, 567.00-567.02, 569.00, 577.01, 590.00-590.99, 595.00-595.02, 597.00, 599.02, 601.00, 604.00, 604.01, 607.30, 611.00, 611.01, 612.01-614.99, 616.00-616.03, 620.00-620.99, 622.00-622.19, 629.40, 630.00-630.09, 635.00-636.09, 645.90-645.91, 670.00-670.09, 678.02, 680.00-680.90, 681.00-682.99, 684.00-684.09, 710.00-710.09, 720.00-720.29, 732.99, 761,00, 763.00, 998.50, 999.30
	ICD-9	001-005X, 008A-F, 010-012W, 014-018X, 020-027X, 030-036, 036C-041X, 073, 076, 078D, J, 790H, 080-083X, 087-088, 090-D, 090F-093X, 095-097X, 098-099A, 100-104, 245A, 254B, 360A, 373B, 375D, 376A, 382A-E, 383A-X, 390-391X, 421A, 461-X, 475, 481-482X, 510-X, 511B, 513-B, 522E, H, 526E, 527D, 528A, D, 540B, 562-B, 566, 567-C, 569F, 575A, 590-X, 597A, 595-D, X, 597W, 599A, 601-D, 603B, 604A, 604X, 607B, C, 608A, E, 611A, 614-F, W-X, 615A, X, 616-X, 634A, 635A, 636A, 637A, 638A, 639A, 646F, G, 647A, B, D, 658E, 659D, 670, 675-B, W-X, 681-682X, 683, 684, 685-686X, 711A, E, 728A, 729E, 730-D, X, 771D, 996G, 998F, 999D
	ICD-10	A00-05.9, A15-16.9, A20-22.7, A22.9-28.9, A30-32.0, A32.7-38, A39.1-49, A50-58, A65-69.9, A70-74.9, A75-79.9, B95-96.8, E06.0, E32.1, H00.0, H01.0, H04.3, H05.0, H44.0, H60.0-60.1, H66.0-66.4, H70.0-70.9, I00-01.9, J01-01.9*, J02.0, J03.0, J13-15.9, J16.0, J20.0-20.2, J34.0, J36*, J39.0-39.1, J85.1-85.3, J86-86.9*, K04.6-04.7, K05.2, K11.3, K12.2, K14.0, K35.1, K57-57.9, K61-61.4, K63.0, K65.0*, K81.0, K85, L00, L01-01.1, L02-02.9, L03-03.9, L04-08.9, M00-00.9, M46.3*, M60.0*, M86-86.9*, N10-12*, N13.6, N15.1, N15.9, N30-30.3*, N30.8-30.9*, N34-34.1*, N39.0*, N41-41.3*, N43.1*, N45.0-45.9*, N48.1-48.2*, N49-49.9*, N61, N70-76.8*, N98.0, O07.0, O07.5, O08.0, O23-23.9, O41.1, O75.3, O85-86.8*, O91-91.1, O98.0-98.2, P23.1-23.6, P36, P37.0, T80.2, T81.4, T82.6-82.7, T83.5-83.6, T84.5-84.7, T85.7, T88.0, Z22.0-22.3
Viral non-CNS infection)	ICD-8	008.80-008.98, 050.00-051.99, 052.01-054.03, 054.05-057.99, 060.00-061.99, 067.00-068.98, 070.01-070.09, 072.00, 072.02-072.09, 074.00-075.01, 075.07-075.09, 078.00-079.10, 079.40-079.99, 099.92, 460.99, 464.01-464.09, 465.99, 470.99-473.99, 480,99, 508.03, 761,20, 761,30
	ICD-9	008H-M, 050-052A, 052W-053, 053B-054C, 054E-055, 055B-056, 056H-057X, 060-061, 065-066, 070-X, 072-A, 072D-X, 074-075, 077-078H, 078W-079X, 279K, 460, 464-465X, 480-X, 487-W, 647F, G, 711F, 771A, B, 790W
	ICD-10	A08-08.4, A60-60.9, A63.0, A90-99, B00-00.2, B00.5-00.9, B01, B01.2-02, B02.2-05, B05.2-06, B06.8-09, B15-19.9, B20-24, B25-26.0, B26.3-34, B97-97.8, J00, J04-06.9*, J10-11.8, J12-12.9, J20.3-20.7, J21.0, O35.3, O98.4-98.5, P23.0, P35, Z21, Z22.5-22.6
Other/unknown non-CNS infection)	ICD-8	006.00-007.99, 009.00-009.98, 084.10-087.99, 099.96-099.99, 110.00-117.99, 120.00-129.99, 130.00-130.10, 130.99, 131.97-131.99, 136.09, 360.00, 380.02-380.09, 381.00-381.99, 384.00-384.08, 420.00-420.09, 421.98, 422.97-422.99, 462.01, 462.09, 463.09, 466.99, 483.99-486.09, 503.00-503.09, 540.00-540.02, 540.04-540.99, 572,99, 686.00-686.98, 761,40, 763.10, 763.98, 778,60
	ICD-9	006-E, 006G-007X, 008W, 009-D, 084-086X, 099E-X, 110-118, 120-129, 130, 131-131X, 132-134X, 135-136X, 370E-F, X, 372A-D, 380B, C, 381A, 382X, 420-X, 421, X, 422, 462, 463, 466-B, 473-X, 483, 485, 486, 490, 491B, 540A, X, 572A, 647C, E, W, X, 680A, 711G-X, 727A, 770A, 771C, E-W
	ICD-10	A06-06.5, A06.7-07.9, A08.5, A09, A59-59.9, A63, A63.8-64, B35-37.4, B37.6-38.3, B38.7-43.0, B43.2-45.0, B45.2-46.0, B46.2-49, B50, B50.8-57.3, B57.5, B58-58.1, B58.3-60.1, B60.8-64, B65-69, B69.1-83.1, B83.3-83.9, B85-89, B99, H10.0, H10.3-10.9, H16.2-16.3, H16.9, H32, H60, H60.3, H65.0, H65.1, H66.9, I30.0-30.9, I33.0-33.9, I40.0, J02*, J02.8-02.9*, J03*, J03.8-03.9*, J16, J16.8, J18-18.9, J20, J20.8-21, J21.8-21.9, J22, J32-32.9*, J35.0, J37-37.1*, J40-42, K35, K35.9, K75.0, L30.3, M46.5, M65.1, M71.1, O98.3, O98.6-98.9, P23.8-23.9, P37.1-39.9, Z22.4, Z22.8-22.9

[1] The Swedish ICD coding systems were used the following years: ICD-8 1969-86, ICD-9 1987-96 and ICD-10 1997-.

[2] CNS=Central Nervous System.

* The additional codes, B95-97.8, determines the infecting organism.

Table S2. Mann-Whitney U-test, p-values of the difference in levels of acute phase proteins among neonates to exposed and unexposed mothers (median, 25th-75th percentile), controls and cases separately. (Study II)

APP	HSV-1								
	Controls			Neonates who will develop non-affective psychosis, ICD-10 F20-29			Neonates who will develop schizophrenia, ICD-10 F20		
	Unexposed (n=403)	Exposed (n=122)	p	Unexposed (n=153)	Exposed (n=46)	p	Unexposed (n=30)	Exposed (n=16)	p
a-2-Macroglobulin (ng/ml)	370 (141-651)	436 (175-703)	ns	321 (76-537)	417 (196-771)	ns	343 (42-536)	336 (146-590)	ns
Haptoglobulin (ng/ml)	6.7 (2.7-21.1)	6.9 (2.8-27.7)	ns	5.8 (2.2-15.9)	6.9 (2.9-17.4)	ns	6.7 (1.9-33.7)	6.2 (1.7-42.4)	ns
C-reactive protein (ng/ml)	0.7 (0.2-1.8)	0.8 (0.2-2.1)	ns	0.6 (0.2-1.1)	0.7 (0.3-1.8)	ns	0.5 (0.1-1.2)	0.4 (0.1-1.2)	ns
Serum amyloid P (ng/ml)	10.6 (5.4-18.1)	11.3 (5.5-17.3)	ns	7.8 (4.7-13.0)	10.0 (5.1-16.9)	ns	9.0 (4.8-13.8)	8.2 (4.5-12.6)	ns
Procalcitonin (pg/ml)	2.3 (0.8-3.6)	2.2 (0.8-3.8)	ns	1.8 (0.4-3.2)	2.5 (0.8-3.8)	ns	2.2 (0.2-4.2)	1.9 (0.3-2.6)	ns
Ferritin (pg/ml)	1410 (280-3020)	1430 (410-2920)	ns	1170 (190-2430)	1870 (680-3830)	ns	1360 (150-2210)	950 (210-2840)	ns
tPA (pg/ml)	3.9 (1.6-6.6)	3.8 (1.8-6.1)	ns	2.6 (0.0-4.3)	3.4 (1.4-6.1)	ns	2.9 (0.4-6.4)	2.6 (0.3-4.4)	ns
Fibrinogen (ng/ml)	6.6 (2.1-19.4)	8.1 (2.4-28.6)	ns	5.7 (2.3-15.6)	7.9 (2.1-26.8)	ns	2.8 (1.3-13.8)	5.6 (2.7-26.8)	ns
Serum amyloid A (ng/ml)	1.7 (0.7-3.6)	2.7 (0.8-3.8)	ns	1.2 (0.5-2.4)	1.7 (0.8-3.3)	ns	1.9 (0.5-2.7)	1.2 (0.3-2.0)	ns

APP	HSV-2								
	Controls			Neonates who will develop non-affective psychosis, ICD-10 F20-29			Neonates who will develop schizophrenia, ICD-10 F20		
	Unexposed (n=135)	Exposed (n=390)	p	Unexposed (n=48)	Exposed (n=151)	p	Unexposed (n=8)	Exposed (n=38)	p
a-2-Macroglobulin (ng/ml)	411 (161-676)	389 (139-694)	ns	330 (115-577)	450 (184-736)	ns	283 (61-494)	628 (427-684)	ns
Haptoglobulin (ng/ml)	6.7 (2.8-27.7)	6.9 (2.5-27.2)	ns	5.9 (2.2-15.2)	7.9 (3.0-20.6)	ns	6.4 (1.7-35.4)	6.2 (4.0-48.3)	ns
C-reactive protein (ng/ml)	0.8 (0.3-2.0)	0.8 (0.2-2.0)	ns	0.5 (0.2-1.2)	0.9 (0.5-1.8)	ns	0.4 (0.1-1.2)	0.6 (0.2-0.9)	ns
Serum amyloid P (ng/ml)	11.1 (5.8-17.4)	10.7 (5.2-19.6)	ns	8.8 (4.6-14.3)	10.9 (6.4-18.6)	ns	7.5 (3.6-12.0)	13.0 (8.4-19.0)	ns
Procalcitonin (pg/ml)	2.2 (0.8-3.8)	2.1 (0.4-3.8)	ns	2.0 (0.8-3.6)	2.0 (0.6-3.2)	ns	2.0 (0.0-3.5)	2.1 (0.8-3.8)	ns
Ferritin (pg/ml)	1450 (390-3060)	1320 (280-2690)	ns	1470 (250-2830)	1480 (600-3770)	ns	860 (100-2120)	3230 (1250-6220)	ns
tPA (pg/ml)	4.1 (1.8-6.5)	3.2 (1.0-5.6)	ns	2.8 (0.5-5.0)	3.4 (1.3-5.9)	ns	2.7 (0.3-3.9)	4.6 (1.9-6.5)	ns
Fibrinogen (ng/ml)	8.2 (2.6-28.5)	5.1 (1.7-16.1)	ns	6.8 (2.3-25.3)	7.1 (1.6-19.0)	ns	3.5 (1.4-26.4)	3.5 (2.8-15.8)	ns
Serum amyloid A (ng/ml)	1.8 (0.8-3.6)	1.6 (0.5-4.0)	ns	1.2 (0.5-2.8)	1.9 (0.8-2.9)	ns	1.2 (0.1-2.0)	2.0 (0.9-2.8)	ns

*=p<0.05, **=p<0.01, ***=p<0.001, ns=non-significant

Table S3. Hazard Ratio (HR) (95% CI) of non-affective psychoses among individuals born in Sweden 1973-85, after hospital admission with various infectious agents during childhood. (Study III)

Type of infection	Hospital admission with infection														
	0-13 years			First year			1-4 years			5-9 years			10-13 years		
	Cases N	Crude HR (95% CI)	Adjusted[1] HR (95% CI)	Cases N	Crude HR (95% CI)	Adjusted[1] HR (95% CI)	Cases N	Crude HR (95% CI)	Adjusted[2] HR (95% CI)	Cases N	Crude HR (95% CI)	Adjusted[2] HR (95% CI)	Cases N	Crude HR (95% CI)	Adjusted[2] HR (95% CI)
Any inf	1114	1.27 (1.19-1.36)	1.10 (1.03-1.18)	300	1.29 (1.15-1.45)	1.05 (0.93-1.18)	586	1.22 (1.12-1.33)	1.05 (0.96-1.14)	290	1.31 (1.16-1.47)	1.17 (1.04-1.32)	157	1.19 (1.01-1.39)	1.09 (0.93-1.28)
Bact inf	240	1.41 (1.24-1.60)	1.23 (1.08-1.40)	47	1.17 (0.88-1.56)	0.99 (0.74-1.32)	92	1.51 (1.23-1.86)	1.30 (1.06-1.60)	52	1.08 (0.82-1.41)	0.97 (0.74-1.28)	56	1.76 (1.35-2.29)	1.57 (1.21-2.05)
Viral inf	443	1.14 (1.04-1.26)	0.99 (0.90-1.10)	108	1.18 (0.97-1.42)	0.98 (0.81-1.19)	236	1.04 (0.91-1.18)	0.90 (0.79-1.03)	117	1.33 (1.10-1.59)	1.15 (0.96-1.39)	33	1.17 (0.83-1.65)	1.01 (0.72-1.43)
Other inf	633	1.31 (1.20-1.42)	1.12 (1.03-1.22)	168	1.41 (1.21-1.65)	1.11 (0.95-1.29)	325	1.32 (1.18-1.48)	1.11 (0.99-1.24)	140	1.41 (1.19-1.67)	1.28 (1.08-1.51)	82	1.06 (0.86-1.32)	1.00 (0.80-1.25)
CNS inf	45	1.38 (1.03-1.86)	1.22 (0.91-1.64)	5	1.06 (0.44-2.56)	0.91 (0.38-2.19)	14	1.27 (0.75-2.14)	1.08 (0.64-1.83)	17	1.36 (0.84-2.18)	1.22 (0.76-1.96)	10	2.14 (1.15-3.97)	1.96 (1.05-3.62)
Non-CNS inf	1089	1.27 (1.18-1.36)	1.10 (1.02-1.18)	299	1.29 (1.15-1.45)	1.06 (0.94-1.19)	576	1.22 (1.11-1.33)	1.04 (0.96-1.14)	278	1.31 (1.16-1.48)	1.17 (1.04-1.32)	150	1.17 (0.99-1.37)	1.07 (0.91-1.26)

[1] Adjusted for male sex, urban birth, parental migration, parental psychiatric history, and hospital admission with other diagnoses.

[2] Adjusted for male sex, urban birth, parental migration, parental psychiatric history, hospital admission with other diagnoses, and infection during previous time periods.

Table S4. Associations between non-affective psychoses and parental hospital admission with infection among individuals born in Sweden 1978-97, Hazard Ratios (HR) and 95% CI. (Study IV)

Mother hospital admitted with infection during pregnancy	*N, Non-affective psychosis/ No diagnose of Non-affective psychosis*	*Basic model*[1]	*Model 1*[2]	*Model 2*[3]	*Model 3*[4]	*Model 4*[5]
		HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
Any infection	117/ 23 667	1.26 (1.05, 1.52)	1.23 (1.02, 1.48)	1.13 (0.94, 1.35)	1.07 (0.89, 1.28)	1.06 (0.88, 1.27)
Bacterial infection	66/ 13 018	1.31 (1.02, 1.66)	1.27 (1.00, 1.62)	1.15 (0.90, 1.47)	1.09 (0.85, 1.38)	1.08 (0.84, 1.37)
Viral infection	18/ 4 041	1.04 (0.66, 1.66)	1.00 (0.63, 1.58)	0.91 (0.58, 1.45)	0.86 (0.54, 1.36)	0.85 (0.54, 1.36)
Other infection	32/ 6 694	1.26 (0.89, 1.78)	1.23 (0.87, 1.74)	1.15 (0.81, 1.63)	1.11 (0.78, 1.57)	1.10 (0.78, 1.56)
0th trimester	7/ 1 424	1.10 (0.53, 2.32)	1.03 (0.49, 2.16)	0.92 (0.44, 1.93)	0.86 (0.41, 1.81)	0.85 (0.40, 1.79)
1st trimester	12/ 3 369	0.91 (0.52, 1.60)	0.88 (0.50, 1.55)	0.79 (0.45, 1.39)	0.74 (0.42, 1.30)	0.73 (0.41, 1.28)
2nd trimester	38/ 8 354	1.15 (0.84, 1.58)	1.11 (0.81, 1.53)	1.02 (0.74, 1.40)	0.96 (0.70, 1.32)	0.95 (0.69, 1.31)
3rd trimester	61/ 11 164	1.42 (1.11, 1.83)	1.40 (1.09, 1.81)	1.29 (1.00, 1.66)	1.24 (0.96, 1.59)	1.23 (0.96, 1.58)
Mother hospitalized with infection up to 5 years before pregnancy	706/ 130 532	1.33 (1.23, 1.44)	1.29 (1.19, 1.39)	1.19 (1.10, 1.28)	1.13 (1.05, 1.22)	1.14 (1.05, 1.23)
Father hospitalized with infection up to 5 years before pregnancy	283/ 64 894	1.09 (0.97, 1.23)	1.06 (0.94, 1.19)	1.00 (0.89, 1.13)	0.97 (0.86, 1.09)	0.97 (0.86, 1.09)

[1] Adjusted for year of birth and sex.
[2] Additionally adjusted for mother or father with psychotic disorder (ICD-10 F20-29).
[3] Additionally adjusted for mother or father with psychiatric disorder (ICD-10 F00-99).
[4] Additionally adjusted for low SES, and any parental inpatient care before or during pregnancy except for treatment of infection or psychiatric care.
[5] Additionally adjusted for urban birth, winter birth, parental age ≥ 35 years, small for gestational age, and parent born outside Sweden